# GENETIC MARKERS IN DIABETES MELLITUS

by

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STATEMENT

This thesis describes the results of research carried out in the Department of Human Biology, John Curtin School of Medical Research at the Australian National University since September 1977, under the supervision of Dr. R.L.Kirk.

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ABSTRACT

The present investigation provides information on the genetics of susceptibility to diabetes. Patients and controls from five ethnic groups have been studied.

Australian Population: Patient series were available from hospital sources in Melbourne and from surveys in three localities in Western Australia (W.A.). Controls were blood bank donors in Canberra and Sydney.

For genetic markers on chromosome 6, the results demonstrate a strong association for the Properdin factor B (Bf) factors $BfF_1$ and $BfS_1$ in the insulin dependent diabetes mellitus (IDDM) patients of Melbourne. Bf $F_1$ is present in 1.0% of controls compared with 6.0% in the IDDM series. This is an age-dependent association which declines at the older ages. The relative risk for $BfS_1$ and $BfF_1$ combined for patients below 10 years of age is 9.4. Another important feature is a significant decrease of BfF factor in the Western Australian non-insulin dependent diabetes mellitus (NIDDM) patients. This points to the importance of the Bf system, not only in IDDM, but also in NIDDM patients.

The complement $C'2$ system also shows a significant association with IDDM in the Melbourne series. A third chromosome 6 marker, the red cell enzyme Glyoxalase (GLO) shows an apparent association by an increase of the GLO 2-2 phenotypes among the NIDDM patients in Melbourne, but this is due to the ethnic stratification of the population.

Among the non-chromosome 6 markers studied there is a significant association of the phosphoglucomutase (PGM)

allele $\underline{PGM}_1^2$ in the NIDDM patients above the age of 70 years.

A multivariate analysis of the genetic markers for the Australian series indicates genetic differences between the various patient series, as well as between these and the control series.

Indian Population: Among the Indian IDDM patients, the distribution of the chromosome 6 marker Properdin B (Bf) differs from that in Caucasian IDDM. Unlike the latter there is an absence of $BfF_1$ in the Indian IDDM patients, but an association with the other rare factor $BfS_1$ (3.2% controls vs 11.4% IDDM) is noted. In the NIDDM series, there is an increase of the BfF factor. Typing of C´2 was not carried out for this series. For red cell enzyme glyoxalase (GLO), an increase in the GLO 2-2 phenotype was noted and like the Australian series this is due to the ethnic stratification within the Indian population.

For the non-chromosome 6 markers there is an increase of $\underline{PGM}_1^2$ allele unlike that observed in NIDDM series of Australia.

Samoan Population: In the NIDDM series from Samoa, for the chromosome 6 markers there is a decrease of the common allele $\underline{Bf}^F$, which is similar to that for the Australian NIDDM series. The C´2 typing was not carried out for the Samoan series. At the GLO locus no significant disturbance in phenotypes or gene frequencies are observed. Among the non-chromosome 6 markers, $\underline{PGM}_1^2$ also shows a decrease in the NIDDM series, similar to that noted in the Australian NIDDM series in patients over the age of 70 years. The other non-chromosome 6 markers do not yield informative

results for the Samoan series.

Nauru Population: For the chromosome 6 marker Properdin factor B (Bf), there is an increase of $Bf^F$ among the NIDDM patients similar to the phenomenon noted in the Indian NIDDM series. The other markers on chromosome 6, C'2 and GLO, were not studied in this population.

Among the non-chromosome 6 markers, there is an increase of the $PGM_1^2$ allele, a reverse trend to that in the Australian NIDDM series. This may be due to the difference in the age structure of the population. A significant association with a non-chromosome 6 marker, $6GPD^C$, was noted in the Nauru NIDDM series.

Pima Indian population: There is no significant disturbance in the distribution of the chromosome 6 markers Bf and GLO phenotypes or gene frequencies in the NIDDM series of Pima Indians. For the C'2 system, an increase of $C'2^2$ allele is noted in the NIDDM series.

Among the non-chromosome 6 markers, there is a significant increase of $PGM_1^2$ allele (8.4%) in the NIDDM series compared to controls. This is unlike the Australian NIDDM series, and may be due to the lower age of individuals in this population. The association of phosphoglycolate phosphatase (PGP) with NIDDM among Pima Indians, which has been claimed by other workers, is not supported by this study.

In conclusion, the study shows the importance of two markers (other than HLA) on chromosome 6 which are informative for indicating the risks of persons carrying the properdin factors $BfF_1$ and complement allele $C'2^2$, for

the IDDM type of disease.

In the NIDDM type of disease, association of the PGM markers requires further study for confirmation.

The other important points highlighted in this investigation are: (1) ethnic stratification within a population may produce artifacts of association with genetic markers, and (2) breakdown of the population into age groups also may be important in revealing the effect of association in particular age groups.

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# Chapter 1

## INTRODUCTION

The prevalence of diabetes ranks it as one of the leading health problems in the world. The notion that it is a disease only of affluent societies is erroneous, since many developing countries also show high prevalence rates of the disease. In fact, it is regarded as a universal problem affecting human societies at all stages of development (WHO, 1980).

### Definition of Diabetes Mellitus

The effects of diabetes are recognised by the state of chronic hyperglycaemia, a consequence either of insulin lack or due to a number of factors which inhibit its action. Since insulin is one of the major regulators of blood glucose concentration, an imbalance leads to abnormalities of carbohydrate, protein and lipid metabolism. As the disease progresses other effects may include characteristic symptoms, such as ketoacidosis, gradual degeneration resulting in disease of capillaries of the kidney and retina, damage to the peripheral nerves and excessive arteriosclerosis (WHO, 1980). For long it was regarded as a single disorder of metabolism, For example, one of the standard medical textbooks defined diabetes as "..... a disorder of carbohydrate metabolism characterized by hyperglycemia and glycosuria associated with a disturbance of the normal insulin mechanism ....." (Bondy 1963). A more recent definition by Fajans *et al.* (1978), however, indicates a broadening of this concept." Idiopathic diabetes mellitus

is a genetically determined disorder of metabolism associated with absolute or relative insulin insufficiency which, in its fully developed clinical expression, is characterised by fasting hyperglycaemia, atherosclerotic and microangiopathic vascular disease and neuropathy". Apart from these essential characteristics Fajans *et al*, (1978) point out that variation in the clinical presentation may occur, such that the disease may be a mild form and without complications.

The basis for separation of diabetes into various types has been greatly stimulated by the discovery of associations between markers such as HLA and islet cell antibodies in some diabetic subjects and not in others. This has led to fresh attempts to classify diabetes on the basis of clinical and laboratory features.

Classification Schemes:

The first attempt at classification was made by a workshop on "Etiology and pathogenesis of insulin-dependent diabetes mellitus" (Philadelphia Workshop 1977) co-sponsored by the National Institute of Arthritis, Metabolism and Digestive Diseases and the Juvenile Diabetes Foundation. The main emphasis of this workshop centered specifically on the juvenile type of diabetes with some reference to non-insulin or maturity onset type of diabetes.

In 1978 the Kroc Foundation sponsored a conference on the "Epidemiology of diabetes and its macrovascular complications" which discussed some of the definitions and schemes of classification (West 1979). These discussions were of

assistance to the National Diabetes Data Group (NDDG) of the National Institutes of Health which also published a report on classification and diagnosis of diabetes mellitus (NDDG, 1979). This classification is the basis for that adopted in 1980 by the World Health Organization (WHO, 1980). Since this scheme is based on contemporary knowledge incorporating the heterogeneity of the syndrome, it will form the basis of this thesis. An outline of this classification, given in table 1.1, will be briefly described below.

Type I: Insulin dependent diabetes mellitus (IDDM), usually characterized clinically by abrupt onset of symptoms, insulinopaenia and dependence on injected insulin to sustain life, and proneness to ketosis. Classically, this type of disease occurs in juveniles and was formerly termed juvenile diabetes. Since it can be recognized and becomes symptomatic at any age, diagnosis on an age basis is abolished. IDDM appears to be heterogeneous in terms of genetic and environmental factors which precipitate the disease (Rotter and Rimoin, 1978).

Genetic determinants are important in most patients, confirmed by the associated increased or decreased frequency of certain histocompatibility antigens (HLA) controlled by genes on chromosome 6 (Nerup *et al*, 1974; Cudworth and Woodrow, 1974).

Type II: Non-insulin dependent diabetes mellitus (NIDDM), frequently presents with minimal or no symptoms referable to the metabolic aberrations of diabetes. Patients with NIDDM are not dependent on insulin for prevention of ketonuria and are not prone to ketosis. They may require insulin for corr-

Table 1.1

National Diabetes Data Group: Classification of Diabetes Mellitus

A. Clinical Classes:

DIABETES MELLITUS

Insulin dependent type (IDDM) Type I

Non-insulin dependent type (NIDDM) Type II

1. Non-obese
2. Obese (NIDDM)

Other types, including diabetes mellitus associated with certain conditions and syndromes:

1. Pancreatic disease
2. Hormonal
3. Drug or chemical induced
4. Insulin receptor abnormalities
5. Certain genetic syndromes
6. Other types.

The classification includes also a set of categories for persons with impaired glucose tolerance but without other clinical signs of diabetes.

Gestational diabetes is listed as a separate category.

B. Classification based on statistical risk

(subjects with normal glucose tolerance but substantially increased risk of developing diabetes)

1. Previous abnormality of glucose tolerance (prevAGT)
2. Potential abnormality of glucose tolerance (PotAGT)

ection of symptomatic or persistent fasting hyperglycaemia if this is not achieved by use of diet or oral agents. Ketosis may develop under stress or trauma. A range of insulin responses to glucose, from low to supranormal, has been found in patients of this class, many of whom do not have fasting hyperglycaemia.

Complications of diabetes, namely macro- and microangiopathy, neuropathy, retinal damage and cataracts, are often associated with Type II or NIDDM. NIDDM is also of a heterogeneous nature and use of age of onset is not recommended (NDDG, 1979). NIDDM has a strong genetic basis, which is evidenced by a more frequent familial pattern of occurrence (NDDG, 1979). Included under this type are families in whom diabetes occurs in children, adolescents and adults with an autosomal dominant inheritance known as MODY and described by Tattersall and Fajans (1975).

Intake of excessive calories leading to weight gain and obesity is probably an important factor in the pathogenesis of NIDDM. Although small changes in weight may be important, NIDDM has been subdivided according to the presence or absence of obesity. 60%-90% of all NIDDM patients are obese, especially noted in the Western societies (NDDG, 1979).

Unlike IDDM, the NIDDM type of diabetes is not associated with HLA types and islet cell antibodies among Caucasians. However, recent reports from South Africa for the Xhosa tribe (Briggs *et al.* 1980) and for Fiji Indians (Serjeantson *et al.*, 1981) have shown association of NIDDM with some HLA antigens in these populations. These will be discussed more fully in a later section.

Other types of diabetes: In this subclass, diabetes forms part of certain other conditions and syndromes which often have many clinical features not generally associated with the diabetic state. Only in some cases the co-occurrence of glucose tolerance and other features may be of some importance aetiologically. In others, it can imply a causal relationship. This class has been further subdivided depending upon the known or suspected aetiologic relationship. (1) It may be secondary to pancreatic diseases or removal of pancreatic tissue, (2) it may be due to endocrine disease such as acromegaly, Cushing's syndrome, pheochromocytoma, glucagonoma, somatastatinoma and primary aldosteronism, or (3) it may result from the administration of certain hormones, drugs and chemicals which cause hyperglycaemia.

Diabetes may also be associated with defects of the insulin receptors which may be caused either by abnormalities in numbers or to affinity of insulin receptors or antibodies to receptors with or without associated immune disorders (Roth *et al*, 1979). As well carbohydrate intolerance is often seen in association with a number of genetic syndromes (Rimoin and Schimke, 1971; Rimoin, 1976). Finally, this class incorporates special types of diabetes found among specific populations and environmental conditions, e.g. diabetes associated with malnourished people (NDDG, 1979).

## Prevalence of diabetes mellitus in the world populations

Historically, diabetes was known to occur in certain populations since ancient times, and original records have been found in the literature from Egypt (Ebers papyrus),

China, Japan, India and from Cappadocia (now called Iraq). More recently, epidemiological surveys have shown diabetes to be universally distributed among all populations. Rates for various parts of the world are summarized from West (1978) for IDDM in Table 1.2 and for NIDDM in Table 1.3. It should be remembered, however, that the rates have been determined by different methods and therefore are not always reliable.

The main points of interest which emerge from the survey of diabetes prevalence rates, as shown in these tables, is that there is a high rate of IDDM among Caucasian populations, ranging from 0.3 in France to 3.7 for 20 year-old conscripts in Australia, whilst in non-Caucasian populations IDDM has a very low rate. By contrast a high rate of NIDDM is commonly found among non-Caucasian populations whilst it is relatively low among Caucasians.

Recent interest has focussed on populations showing high prevalence rates of diabetes of the NIDDM type and also in situations where there is a striking contrast in diabetes prevalence rates among populations inhabiting the same geographical area.

The first focus of attention permits a discussion of possible environmental factors influencing the prevalence of diabetes, whilst the second suggests the possibility of environmental-genetic interaction. This possibility will be discussed below after examining environmental factors alone.

Among the populations exhibiting very high prevalence rates are the Micronesians of Nauru (Zimmet *et al*, 1978), Pima Indians of Arizona (Bennett *et al*. 1971), Natal Indians of South Africa (Jackson, 1970) and Aborigines of

Table 1.2 Prevalence rate of juvenile onset type of diabetes mellitus, or IDDM, in different populations.

Note: Ref. for investigations listed in table will be found in West 1978 (except where indicated +).

| Investigator & Year | Population | Age | Rate/ 1,000 | Source |
|---|---|---|---|---|
| Palumbo *et al.* (1976) | Rochester Minn. School Children | 5-18 | 1.0 | Survey of clinical records |
| Sultz *et al.* (1968) | Erie County N.Y. | 0-16 | 0.6 | Counting of known cases |
| Falconer *et al.* (1971) | Edinburgh | 10-19 | 0.93 | Diagnosed cases |
| Wadsworth & Jarrett (1974) | Britain | 11 | 0.1 | Diagnosed cases in cohort |
| Lestradet (?) | France | 0-19 | 0.3 | Registry (insulin treated only) |
| Kruger (1965) | Schwerin E. Germany | 1-9 | 0.16 | Urine screening |
| Pinelli (1976) | Venice, Italy | 0-13 | 0.26 | |
| Holmgren *et al.* (1974) | Vasterbotten County, Sweden | 0-15 | 2.2 | Known cases |
| Christau *et al.* (1976) | Denmark | 0-29 | 0.7 | Registry |
| Proust & Smithurst (1968) | Australia | 20 yrs (males) | 3.7 | Interview and registration for Nat.Service |
| Miki & Maruyama (1970) | Japan | 0-15 | 0.025 | Survey estimate of diagnosed cases |
| Sathe (1973) | India | - | Rare | |
| Culloch (1962) Seftel (1964) | Primitive blacks of several areas and in Chinese Indians & Malays of Singapore | - | Rare | |
| Hai *et al.* (1965) | Vietnam | - | Rare | |
| Wang (1937) | Chinese | - | Rare | |
| Cassidy (1967) | Indians & Melanesians of Fiji | - | Uncommon | |
| Belcher (1970) | Ethiopia | - | Uncommon | |
| Tarrett & Keen (1975) | Malta, Ceylon Cook Island Maoris & Trinidad blacks | - | Uncommon | |
| Zimmet *et al,* (1978) | Polynesians & Micronesians | - | Rare | |
| Bennett *et al.* (1971) | Pima | - | Rare | |

Table 1.3 Prevalence of Adult onset diabetes, or NIDDM, among different populations

| Investigator & Year * | Population | Age | Rate in % |
|---|---|---|---|
| Wilkerson & Krall (1947) | Massachusetts<br>Oxford | Adults | 1.1 |
| Palumbo *et al.* (1976) | Rochester | Over 40 | 4.1 |
| Redhead (1966) | England (Newcastle) | Over 30 | 0.5 |
| Nilsson *et al.* (1964) | Sweden (Kristianstad) | Adults | 2.7 |
| Welborn *et al.* (1968) | Australia<br>Busselton)<br>Cunderdin) | Adults | <br>0.9<br>1.3 |
| Cardonnet & Nusimovich (1968) | Argentina<br>Rosaria | Adults | 2.9 |
| Mateo de Costa *et al.* (1973) | Cuba, Artmisia )<br>Rural areas ) | Adults | 0.7<br>0.2 |
| Sasaki *et al.* | Japan, Yao<br>Rural | over 39 | 1.3 |
| Mouratoff (1967) | Eskimo | all ages | 0.03 |
| Mouratoff (1969) | Alaska; Athabascan | all ages | 0.02 |
| Stein *et al.* (1965) | Cherokee Indians | all ages | 10 |
| Reinhard & Greenwalt (1975) | Papago Indians<br>Arizona | all ages | 5.3 |
| Bennett *et al.* (1971) | Pima Indians | above 30 | 40 |
| West (1974) | Oklahoma Indians | Adults | 10 |
| Wise *et al.* (1970) | Australian Urbanized Aborigines | over 20 | 8 |
| Hingston & Price (1964) | Papua Rural | Adults | 0 |
| Zimmet *et al,* (1976) | Nauru | over 14 | 34 |
| †Marine *et al.* (1969) | South Africa -<br>Indians<br>Malay<br>Bantu | over 15 | <br>10.4<br>6.6<br>3.6 |

* References for investigations listed in table will be found in West 1978 (except where indicated †)

South Australia (Wise *et al*, 1976).

These high rates seem to coincide with a change from a traditional to a 'Western' life style, associated with a high carbohydrate diet, often including excessive alcohol intake and a reduced output of exercise, thus encouraging obesity.

## Environmental factors influencing prevalence of diabetes mellitus.

### Diet, obesity and exercise

The possibility that risk of diabetes is related to carbohydrate consumption has been frequently suggested. This hypothesis was based on several considerations. Although the ingestion of fat and protein also stimulates pancreatic beta cell function, the ingestion of carbohydrate presents a challenge to beta cells which is stronger, more direct and more immediate than when protein or fat are ingested alone. Blood glucose levels are higher immediately after ingestion of carbohydrates than after protein or fat. Improvement in diabetes is seen when the carbohydrate intake is restricted.

It was once considered that rates of diabetes would reflect the dietary intake of the population, particularly the intake of sugar. Although supporting evidence does come from some sources, equally strong evidence against the hypothesis has been presented, such as the observation that West Indies cane cutters rarely experience diabetes despite the high dietary sugar intake (Brigham 1868). High sugar-consuming Hawaiians and Cubans also had low diabetes death rates, although the rich sweet-eating Bengalis of India did show a high rate of the disease (West 1978).

A very important issue is whether the increase in serum insulin levels is related mainly to obesity itself or to associated factors such as indolence or the increased levels of food intake. Probably all three factors (obesity, overeating and inactivity) are significant (Grey & Kipnis 1971). Himsworth (1935) cited evidence that high carbohydrate regimens might increase sensitivity to insulin. Perhaps Africans, who are obese, are less hyperinsulinaemic than Americans who eat more fat and less carbohydrate (West 1978). Diet is a variable factor in different ethnic groups, but it is not the only index of variation in clinical features. For example, Rimoin & Schimke (1971) have shown variation in ketosis occurrence in some countries when fat and carbohydrate consumption is the same (table 1.4).

For more than a century the dilemma of obesity causing diabetes or diabetes being the cause of obesity has been under intensive investigation. Vallence Owen (1962) and Mirsky (1949) are of the opinion that obesity may be secondary to the diabetic diathesis. No available evidence suggests any causal role for hyperglycaemia in obesity. On one hand, there is a debate of obesity and risk to diabetes and, on the other hand, that of leanness. In one study in the Gondar area of Ethiopia (Belcher 1970) the incidence of diabetes decreases sharply with age in the very lean population. While leanness is not considered to confer a lesser or greater risk, obesity is considered a contributing factor in the manifestation of diabetes. The reported incidence shows a relatively lower rate among the lean or non-obese

Table 1.4

Ethnic variation in diabetes mellitus.
(taken from Rimoin and Schimke 1971).

| Ethnic Group | DIETARY | | Ketosis | Vascular Complications |
|---|---|---|---|---|
| | Fat | Carbohydrate | | |
| European | high | high | common | common |
| Pima Indians | " | " | rare | " |
| Seneca Indians | " | " | " | " |
| Eskimo | " | low | " | rare |
| Japanese | low | high | " | uncommon |
| Indian | " | " | " | common |
| South African Zulu | " | " | common | rare |
| South African Indian | " | " | rare | very common |
| Ashkenazi Jews | High | high | common | common |

group (Rao *et al*, 1966). Gupta *et al*, (1976) have shown diabetes to be 1.0% in thin persons and 9.3% in fat persons. Similar conclusions from other sources are compatible.

Evidence is set forth that much of the family aggregation of NIDDM is attributable to the family aggregation of obesity. This familiality of obesity has both genetic and non-genetic determinants. Observations of Baird (1973) illustrate the inter-relationships of the familiality of obesity and diabetes. Rates of diabetes were determined by testing siblings of obese and non-obese diabetics. In obese siblings of non-obese diabeticsthe rate of diabetes was highest, while in the non-obese siblings of obese diabetics these rates were lowest (West 1978). Based on studies of populations such as New Zealand Maoris (Prior 1962), Hawaiians (Sloan 1963), urbanized Australian Aborigines (Wise *et al*, 1970) and Sumo Wrestlers of Japan (Kuzuya *et al*, 1975), it is suggested that obesity may precipitate diabetes in individuals genetically susceptible to diabetes since, in other populations when obesity is rare, diabetes is rare (West 1978). The debate, however, can only be resolved by more detailed studies.

Inactivity is known to lead to diabetes, although the short and long-term effects of exercise on carbohydrate metabolism are incompletely understood (West 1978). It has been demonstrated that an inactive life impairs glucose tolerance (Altman *et al*, 1969) and that it is improved with physical exercise (Bjorntorp *et al*, 1977). An example of this can be seen among some tribes, such as Broayas of the Sahara where diabetes is rare and this is attributed to the

high levels of energy expenditure (Rubinstein *et al*, 1969).

Age and Stress

The important relationship between age and diabetes, although indicative of the physiological status of individuals, suggests either direct effects of aging itself or increasing susceptibility due to decreased resistance to infectious agents. Alternatively, it could reflect only secondary relationship with the disease.

Stress is reported to have some influence in precipitating one form of diabetes. Claude Bernard's (1855) experiments showed central nervous system lesions to produce diabetogenic effects. Interestingly, however, during World War I, despite stress of maximum degree, glycosuria was observed in only 2 of 40,000 American soldiers in a hospital centre in France. Similarly, the profound anxieties of civilian populations in Nazi-occupied territories during World War II were attended by substantial declines in the incidence of diabetes (Joslin *et al*, 1933).

Viral aetiology

One of the strongest suspected environmental components in the case of IDDM is virus infection. Viral suspects are many, but the main weakness to the hypothesis is the non-isolation of the exact virus responsible. Some of the viruses implicated in diabetes include mumps virus (Craighead 1975), Coxsackie B (Kibrick and Benirschke 1958), cytomegalovirus (Cappell and McFarlane 1947) and Rubella (Forrest *et al*,

1971).

Animal models, particularly in mice, have shown that a picorna virus-like coxsackie B and FMDV can produce a diabetes mellitus-like syndrome (Craighead and McLane, 1968; Boucher and Notkins 1973) characterized by polydipsia and polyphagia.

Mechanisms are known by which virus infections may trigger the subsequent development of immunological events in the pancreas and other organs, progressing long after the acute infection (Nakhooda *et al*, 1977). The mechanism outlined by Freytag in 1974 shows the possibility that there can be a loss of immunologic tolerance so that the pancreatic islets are at risk to additional pancreatotropic viruses or to an ongoing smouldering, auto-immune process long after the initial infection subsides, leading to clinical diabetes mellitus (Rayfield and Seto 1978).

An added source of evidence for viral aetiology comes from the study by Gamble and Taylor (1969). A correlation between the variations in incidence of Coxsackie B4 infection and the number of new cases of insulin-dependent diabetes was observed. Among the insulin-dependent diabetics in the age range 0-19 years, the lowest incidence was in June, with an increase in early summer, to a broad peak about October. There was a progressive decline until the following summer, supporting Adams'observations in 1926 (Gamble and Taylor 1969). There was no significant seasonal variation in the number of cases with onset before 5 years, but in the age group 5-16 the rate of onset in winter was more than twice as common as in summer (Bloom *et al*, 1975). In data by age of onset and sex differences, only males aged 10-19

years indicated a significant seasonal variation in incidence (Gray *et al*, 1979). Evidence of diabetic cases (IDDM) as a consequence of mumps infection have been reported since the last century (Stang 1864; Harris 1899) such that a direct causal relationship was suggested. Since then evidence for mumps preceding diabetes has been accummulating (Menser *et al*, 1978; Craighead 1975).

In some patients with juvenile diabetes, especially early in the course of the disease, inflammatory cells have been observed in the islets of Langerhans (Gepts 1965). The number of insulin-producing beta cells is decreased and exogenous insulin is generally required. These features and the seasonal variation in the incidence of IDDM give support to the hypothesis that viruses are one cause of IDDM (Craighead 1975; Notkins 1977; Rayfield and Seto 1978).

Antibodies to coxsackie type B4 virus was more often found in diabetics than in controls in the same age group (Gamble *et al*, 1973). High titre of neutralizing antibodies to a virus related to a diabetogenic variant derived from coxsackie B4, was obtained. Innoculations of mice with the human isolate produced hyperglycaemia, inflammatory cells in islets of Langerhans and beta cell necrosis. Staining revealed viral antigens in beta cells, suggesting that this IDDM case was virus induced (Yoon *et al*, 1979).

The age-based correlation is known only for the coxsackie B4 viral incidence. Significance of infection in this age group can be due to a number of possibilities as suggested by Gamble (1976). Most viruses have their highest incidence at the age of 5 to 6 years, the infectivity of the virus may be low with a peak at 12-15 years. There may be a long period of latency between infection and development of overt diabetes. A single pancrea-trophic infection may produce sub-diabetogenic damage and several such infections may be required to produce overt diabetes. Alternatively, the infection may depend upon the strain of the virus, the passage history of the virus and the genetic background of the host (Notkins 1977).

Genetic factors influencing prevalence of diabetes mellitus

Having considered above the influence of environmental factors on the prevalence of diabetes, it was noted in the sections under diet and obesity that there is not always a high correlation between increased diet and prevalence of diabetes (table 1.4). This suggested to some investigators (Zimmet 1979; Wise *et al*, 1976 and Jackson 1970), that environmental factors are not responsible alone for the manifestation of diabetes. Each ethnic group, therefore, may have a different predisposition to diabetes.

Two types of studies indicate the importance of this genetic/environmental interaction. The first is a comparison of prevalence rates among populations in the Pacific region - Melanesians, Polynesians and Micronesians, who have recently undergone a change in life-style to a western dietary and activity patterns. According to Zimmet and Kirk

(1979) diabetes is rare in Melanesians, even when they become westernized but high diabetes prevalence rates have been reported in urbanized Polynesian and Micronesian populations. Diabetes is rare in Polynesians and Micronesian populations who have maintained a traditional life-style. They conclude, therefore, that the latter two groups may have a genetic susceptibility to diabetes which has been unmasked by the change from traditional to western life-style, in comparison with the former who, despite similar circumstances, show a low rate of diabetes.

The second type of study is concerned with the expression of diabetes in inbred strains of mice. Several distinct recessive genes have been detected in mice which lead to a diabetes-like syndrome in the homozygous animal. Coleman and Hummel (1973)have shown that two of these recessive genes, *ob* and *db*, result in an identical syndrome when incorporated into strains with the same genetic background. However, the *db* gene on a BL/Ks background has a more severe diabetes-like condition with less pronounced obesity than the *db* gene on a BL/6 background. Thus the same genes may vary in their interaction with the environment when placed in a different general genetic background. Another valuable example is provided by the Japanese KK strain and New Zealand NZO strain of mice (Herberg and Coleman 1977). Both the KK and NZO strains show moderate hyperglycaemia and hyperinsulinaemia when allowed to feed *ad lib*. However, if dietary calories are restricted the KK mouse returns to normal, but the NZO mouse never loses its hyperglycaemia and

remains moderately obese. These two distinct forms of genetic susceptibility to diabetes in the mouse demonstrate clearly the way in which they may differ in the response to changes in dietary patterns. Such differences could well be true for various ethnic groups in man. The following section will briefly consider the different approaches used in the genetic investigations in an attempt to uncover the mechanism of inheritance for the disease manifestation.

## Previous studies of genetics in diabetes mellitus

The earliest record which exists suggesting a genetic basis and difference in types of diabetes, comes from Sanskrit translations. It is reported, by early Hindu physicians, that 'a person suffering from congenital Prameha (diseased urine) owing to the fact that his birth from a father afflicted with Mahumeha (honey urine) cannot be cured for the primary defect in the seed'. The disease was due to two causes, one, attributable to use of injudicious diet and, the other, due to defect in the seeds of one's parent (Sushruta Samhita, cited in Simpson 1976). Rondoletius (1574) also reported the disease to be hereditary in nature.

In more recent studies, pooled family data has been examined for the best fit to Mendel's ratios of dominant, recessive and X-linked inheritance and analyses have utilized sib/parent and proband/sib incidence data to calculate the genetic pattern.

Data on 1000 diabetic pedigrees led to the conclusions that mild mature onset diabetes was controlled by a dominant

gene and early onset diabetes by a recessive gene, and that this was due to independent genes and these were not alleles, distinguishing between two types of diabetes (Cammidge 1928; 1934).

Mendelian expectations were applied to the data of offspring of parents, neither one or both of whom were affected. The proportions obtained approximated values for recessive inheritance (Pincus and White 1933; Steinberg and Wilder 1952b) and further support for recessive inheritance came from Allan (1933) and Hanhart (1939). One study proposed X-linked inheritance (Penrose and Watson 1945) and received support (Barker *et al*, 1951) but was later altered to an autosomal recessive hypothesis.

From a study of 222 probands, without distinction into ages, however, a dominant gene with incomplete penetrance was proposed (Levit and Pessikova 1934; von Kries 1953). These conclusions were based on the fact that the frequency of diabetes among the parents of probands was about equal to that among the sibs. Young diabetics were more likely to have diabetic relatives than the older onset types, and thus early onset type of diabetes was due to a homozygous condition and late onset type due to a heterozygous condition of the gene. Therefore, more sibs of the proband should be diabetic when onset is early, than when it is late and this frequency would not be influenced by the presence or absence of diabetes in a parent ( Harris 1949; 1950). This was not, however, true for other cases (Thomson and Watson 1952; Steinberg and Wilder 1952b).

The attempt to estimate frequency of diabetes from the age of onset among parents was not successful due to the variable nature of diabetes and its mode of inheritance. Despite the lack of agreement on a clear-cut genetic basis for diabetes, family studies provide an empirical basis for estimating the risks of predisposition to the disease, particularly with respect to age of onset. Results from a study of 100 pairs of parent and child diabetics, showed that the average age at onset decreases 20 years per generation and that a child would rarely, if ever, become diabetic at a greater age than that when his parents became affected. This was known as 'anticipation' (Woodyatt and Spetz 1942). Since this was a statistical phenomenon and not a biological one, support was meagre (Steinberg and Wilder, 1950; 1952a).

Later methods were developed to improve estimation, such as K-ratio (Penrose 1953) used to estimate the familial/population frequencies of diabetes in the first degree relatives (Edwards 1960). In families of (IDDM) juvenile onset diabetes corrected for age by weighted means from the different age groups, familial incidence was much higher than incidence in the general population (Simpson 1962). Although, in the earlier data, the first degree relatives of a clinical subgroup (early onset, insulin dependent (IDDM))ketotic diabetes did not fit an autosomal single gene hypothesis, it was close to that expected for multifactorial inheritance. Based on the multifactorial inheritance of diabetes mellitus Falconer

(1965) used a quantitative approach to study the genetics of diabetes. This involved the use of 'heritability of liabilities'. The method developed, utilised data on the incidence of diseases to answer the question of relative importance of heredity and environment.

The degree of genetic determination which, in the study of human populations is called heritability, is the fraction of variation between individuals which causes some to be affected and others not. This was applied to obtain an estimate of correlation or regression coefficient.

'Liability' was referred to as a hypothetical attribute that gave a graded scale of the degree of affectedness or of normality. This term was preferred to 'susceptibility' because it expressed the susceptibility and whole combination of external circumstances which makes an individual more or less likely to develop the disease, whereas susceptibility implies the innate tendency as distinct from the external circumstances (Falconer 1965).

Despite the sophisticated statistical approach, the method was not satisfactory in determining the mode of inheritance for diabetes mellitus.

Analysis by complex segregation among families was used to discriminate between the hypotheses of two allele single-locus inheritance and multifactorial inheritance. Three situations were considered using age of onset for the distinction into early onset, middle and late onset diabetes. In the IDDM (early onset diabetes) the heritability estimated from the multifactorial model was too high and indicated a

major gene action (Goodman and Chung 1975) whereas, when the families were divided into IDDM and NIDDM types, based on insulin treatment in the proband, for IDDM no evidence for a major locus was obtained (Zavala *et al*, 1979). However, for NIDDM or middle and late onset cases, the data fit both a single locus model and a multifactorial model (Goodman and Chung 1975). In some cases of NIDDM, a suggestion of recessive inheritance was indicated and, in others, a strong evidence for a major locus was noted (Zavala *et al*, 1979).

Attempts to identify the 'diabetic genotype' were encouraged by use of glucose tolerance tests as an index (Fajans and Conn 1954; Taylor *et al*, 1967; Köbberling 1969). For a single gene hypothesis one would expect bi-modality in the distribution of blood sugar values among first degree relatives. However, a continuous distribution was obtained with no clear-cut division into normal and affected individuals (Thomson 1965). Since impaired glucose tolerance had a much higher frequency among the relatives of diabetics than in controls, an estimate was made to predict the risk of diabetes in children, based on age.

Different values were assumed by different authors. In some cases it was estimated that 100% of children would have impaired glucose tolerance if they lived long enough (West 1960; Post 1962). In other cases it was assumed 30% would be affected by the age of 85 years (Kahn *et al*, 1969) and Tattersall and Fajans (1975) expected 60% of children to be affected by the age of 60. These estimates, however, do not contribute to the exact knowledge of the

mode of inheritance. Family studies of GTT in different ethnic groups gave fits to various genetic hypotheses. Among the Pima Indians of North America, where a high frequency of impaired glucose tolerance prevails, a bimodal distribution of glucose levels was obtained. Steinberg *et al*, (1970) using a maximum likelihood method of classifying genotypes from a bimodal distribution showed a fit to recessive inheritance. Data on Seminole Indians of Oklahoma, however, with a similar analysis gives fits to both recessive and dominant modes of inheritance. But the Seminole Indians in Florida did not fit either hypothesis (Elston *et al*, 1974).

Family data on GTT among Caucasians from the U.S., Britain and Japan (Neel *et al*, 1965; Thomson 1965; Mimura *et al*, 1964) fit a multifactorial hypothesis. On the basis of a tolbutamide tolerance test on parents of early-onset patients, it was concluded that parents were heterozygous and children homozygous for a gene at the same locus (Braunsteiner *et al*, 1966), a conclusion similar to that of Harris (1950). Thus the results from GTT studies do not give a consistent explanation of the mode of inheritance.

Besides studies of families, population data can be used to distinguish whether a trait is autosomal dominant or recessive. This has been demonstrated in one such study by MacDonald (1980). The frequencies of IDDM are compared in two populations in which there has been a unidirectional influx of genes from the gene pool of one i.e. U.S. Caucasians into that of the other i.e. Black U.S. populations and this

proportion of genes is estimated to be about 20% (Reed 1969).

Accordingly, if IDDM is inherited in a recessive manner then its frequency in the U.S. Black population should be 1/25th of the frequency in Caucasians. However, from the results of Macdonald's (1980) studies it is seen that the ratio of the frequency of IDDM in U.S. Blacks to that in Caucasians is of the same order as the ratio of Caucasian genes in the Black population and this data gives a better fit to a dominant mode of inheritance than a recessive one.

In response to the above hypothesis another study (Rotter and Hodge, 1980) showed that the data can also be explained by a three allele model taking into account the heterogeneity within IDDM. More data may perhaps enable assessment of the mode(s) of inheritance within IDDM more clearly.

Other studies have utilized data on destruction, production and rate of release of insulin among patients. One study showed that a high level of synalbumin, the insulin antagonist, among diabetics was dominantly inherited (Vallence Owen 1966). In another study, it was suggested that NIDDM patients were heterozygous for a gene for increased synalbumin and IDDM patients were homozygous for the same gene (Ehrlich and Martin 1966). These results were, however, not reproducible.

Twin studies

Evidence of familial aggregation of diabetes mellitus suggested the role of genetic factors in susceptibility for

the disease. This was further recognized on the basis of studies on twins whereby a genetic component was demonstrated. Since twins may be monozygotic (MZ), sharing the same genetic components and environment, the influence of genetic factors will be stronger as compared to that of ordinary sibs within a family. Dizygotic (DZ) twins, on the other hand, share a similar environment but not all the genetic components.

Early work on diabetic twins by May (1914), Curtis (1929) and Watson (1934) demonstrated the importance of the genetic component in diabetes through a difference in the concordance of diabetes between monozygotic and dizygotic twins. The concordance rate in monozygotic twins was greater than in the dizygotic twins. Then Bergh (1938) reported on 46 MZ and 87 DZ twins, of which 36 MZ and 50 DZ could be studied clinically and also with an oral glucose tolerance test. Clinical concordance was observed in 17/36 MZ and 9/50 DZ twin pairs. Concordance after glucose load was found in 30/36 MZ and 18/50 DZ twin pairs. No discordance was seen after glucose load in MZ twins aged over 43 years. This was interpreted as being due to a complete manifestation of the hereditary trait, i.e. 'absolute heredity' in older MZ twins. The clinical concordance rates in diabetic twins given by various authors is presented in Table 1.5.

Table 1.5

Clinical concordance rates for diabetic twins.
(taken from Langenbeck and Jörgensen 1976)

| Authors | | Monozygotic T. | Dizygotic T. |
|---|---|---|---|
| Then Bergh 1938 | | 17/36 | 9/50 |
| Lemser 1938 | | 9/12 | 3/14 |
| White and Pincus 1946 | | 16/33 | 2/63 |
| Harvald and Hauge 1963 | | 36/76 | 22/238 |
| Gottlieb and Root 1968 | | 9/30 | 2/60 |
| | Mean | 87/187 | 38/425 |
| | | = 46.5% | = 8.9% |

In juvenile IDDM familial incidence of the disease is higher among concordant MZ twins than in discordant twins (Tattersall and Pyke 1972) confirming Luxenberger's expectation (1935). This implies a predominantly exogenous cause among the discordant MZ twin pairs.

The question raised by discordance of twins, in the IDDM cases, may be related to the time between the diagnosis of the first twin and the development of diabetes in the other twin. Most concordant twins develop diabetes within a time span of 3-10 years of each other. However, in these studies the discordant pairs have remained so even beyond 32 years. The possibility of the discordant twins becoming concordant over a period of longer time (Rosenthal *et al*, 1976) did not receive support (Pyke *et al*, 1976). Therefore, it was concluded that early onset twin pairs will remain discordant, perhaps indefinitely, and the unaffected identical twin is not always destined to become a diabetic (Pyke 1977).

The IDDM twins were investigated for HLA antigens (to be discussed more fully in the next section) and the relative risk for HLA-B 8 positive individuals was 1.5, whereas, for HLA Bw15 = 3.5. Further analysis revealed an increase of Bw15 antigens in both concordant and discordant twins whereas B8 was increased only in concordant pairs. No HLA association was seen among the NIDDM twin pairs (Nelson *et al*, 1975).

One study of identical triplets showed triplet A became diabetic at age 13; triplet B, 8 years later, while C remained non-diabetic, showing normal glucose tolerance tests 11 years later. HLA-B15 was present in all three. The two diabetic triplets showed capillary basement membrane thickening but this was not true for the normal one (Ganda *et al*, 1977).

It can thus be concluded that the twin studies have been important in indicating the different genetic contribution to the susceptibility to the two major types of diabetes. From the above discussion it is noted that concordance rates for IDDM among identical twins is approximately 50%, even after a sufficient time lapse between the onset and diagnosis of the disease in the co-twin of the proband. This suggests that, for IDDM, environmental factors are very important in its aetiology, although genetic factors are also involved. The possible environmental factors may be biochemical in nature, such as the insulin response to glucose infusion, a trait which shows discordance in MZ twins (Cerasi and Luft 1967; Pyke and Taylor 1967; Gottlieb and Root 1968) or due to infection by virus

(Gamble and Taylor 1969; Notkins 1977).

By contrast to the results in IDDM, twin data for NIDDM suggests a very strong genetic component. This is evidenced by Then Bergh's (1938) study, whereby identical twins above the age of 43 are 100% concordant for abnormal glucose metabolism. This phenomenon was further supported by another study of Pyke (1977) where in his large series, over the age of 45 years all twins are concordant for NIDDM, although he points out that other workers have found a few discordant twins with NIDDM.

These observations do not deviate significantly from that expected under recessive inheritance hypothesis. According to Steinberg (1959) the individuals with a genetic susceptibility (which he determined on the basis of affected relatives present in a pedigree) will eventually become diabetic in their life span. Although Steinberg does not clearly state the type of diabetes, this mechanism could apply for the NIDDM type of diabetes due to a long natural history of the disease. However, careful studies are still necessary to examine whether the hypothesis can be accepted or rejected.

## Present status of genetic markers in diabetes mellitus

This section will be discussed in two parts - A and B. Part A will deal briefly with HLA markers in diabetes mellitus and Part B with other, non-HLA markers.

Part A

HLA associations with diabetes mellitus

The Major Histocompatibility Complex in man consists of at least 4 loci (A, B, C and D). The A, B and C loci were detected first using serological methods. The fourth locus, D, was designated initially on the basis of reactions in mixed leucocyte cultures (MLC). More recently, however, D locus antigens have been detected by serological tests against B lymphocytes. The locus controlling these antigens has been termed DR and it is very close to or identical with the D locus controlling MLC reactions.

From the analogous studies in mice and monkeys, the MHC system has been found to control specific immune responsiveness to certain antigens (Benacerraf 1974; McDevitt and Benacerraf 1969). The fact that nearly all associations so far observed between diseases and HLA are stronger with B or D series antigens than with A and C series antigens has been taken as evidence in favour of the existence of immune response (Ir) genes located between the B and D loci.

In an early study no HLA associations with IDDM were observed by Finkelstein *et al.* (1972). However, in a sample of unrelated Caucasian patients a slight increase in the B locus antigen, Bw15 was noted by Singal and Blajchman (1973). Following this, reports from two independent sources, almost simultaneously, demonstrated significant association of IDDM with HLA-B8 and Bw15 (Nerup *et al*, 1974; Cudworth and Woodrow 1974). Later a strong

association of the HLA-D locus antigen Dw3 was noted (Thomsen *et al*, 1975). A brief review of association with HLA antigens will be considered, details of which are found in reviews by Cudworth (1978) and those in Creutzfeldt *et al*, (1976).

HLA-A locus antigens in IDDM

The frequency of HLA-A antigens in patients below 30 years of age indicate relative risks (RR) ranging from 1.61 for A1 to 0.47 for A32 in Caucasians (table 1.6). This increase is secondary to the strong A1B8 linkage disequilibrium (LD). This is a phenomenon whereby, despite crossing over between the various loci of HLA, some HLA genes tend to occur together significantly more frequently in the same haplotype than would be expected under the assumption of equilibrium (Svejgaard *et al*, 1975). Similarly for A2, the increase is due to LD with B15.

HLA A11 remains significantly decreased over all populations showing no heterogeneity between the results (Cudworth 1978; Nerup *et al*, 1977). Reports from other sources (Svejgaard 1974; Seignalet *et al*, 1975) also showed a similar decrease of HLA A11.

HLA-B locus antigens in IDDM

Table 1.7 shows the results obtained from several sources. In each case, an increase in the RR values of antigens B8, B15 and B18, and a decrease of B7, is noted (Cudworth and Woodrow 1976; Nerup *et al*, 1977; Ludvigsson *et al*, 1977).

Table 1.6

HLA-A locus antigens in IDDM

| HLA-A antigens | Cudworth 1978 | Nerup *et al.* 1977 |
|---|---|---|
| | RR | RR |
| A1 | 1.61 | 1.32 |
| A2 | 1.38 | 1.15 |
| A11 | 0.31 | 0.56 |
| A32 | 0.47 | 0.51 |

Table 1.7

HLA-B locus antigens: Relative risk values given by different studies

| HLA-B | Cudworth 1978; Cudworth & Woodrow 1977 | Nerup *et al.* (1977) | Svejgaard & Ryder (1977) | Ludvigsson *et al* (1977) |
|---|---|---|---|---|
| B8 | 2.63 | 2.42 | 3.1 | 2.37 |
| B15 | 1.85 | 1.89 | 2.1 | 2.03 |
| B18 | 2.26 | 1.65 | - | - |
| B40 | 1.30 | 1.32 | - | 1.60 |
| B7 | 0.40 | 0.56 | - | 0.46 |
| +B8 B15 | 5.4 | - | 9.8 | - |
| +B8 B40 | 6.87 | - | - | - |
| +B8 B18 | 2.52 | - | - | - |

In the Copenhagen data, B18 has a lower RR value of 1.65 compared to the Liverpool data of 2.26. HLA phenotype combinations have provided interesting high risk values for B8 + B15; B8 + Bw40 and B8 + B18 with 5.40, 6.87 and 2.52 respectively. Svejgaard and Ryder (1977) using combined data showed a RR = 9.8 for B8 + B15.

HLA-C locus antigens in IDDM

A significant increase in the frequency of the Cw3 antigen has been observed. The relative risks for IDDM conferred on individuals with this antigen in two studies is 2.2 and 2.3 (Ludwig *et al*, 1977; Solow *et al*, 1977).

HLA-D locus specificities in IDDM

A stronger association with the HLA-D locus specificities than with the B locus antigens has been demonstrated by several investigators and this led to the suggestion that the 'diabetogenic' gene(s) may be closer to the HLA-D locus than to the A, B or C loci.

The relative risks for the two main D and DR specificities, from studies on Europeans, are given in table 1.8a and b. It will be noted that the RR values for DW3 and DW4 are higher than the corresponding values given by Svejgaard *et al*, (1977) or by Platz *et al*, (1977) particularly for DW3. Similar high RR values for the serologically determined antigens, DRW3 and DRW4 have been reported by many groups of workers (Jeannet *et al*, 1977; Svejgaard *et al*, 1980; Deschamps *et al*, 1979; Serjeantson *et al*, 1980) and a strong negative association

Table 1.8a

Relative risk values for HLA-D and DR locus specificities and IDDM given by different authors

| HLA-D Specificities | Thomsen et al. (1975) | Svejgaard et al. (1977) | Platz et al. (1977) |
|---|---|---|---|
| Dw3 | 6.4 | 3.8 | 3.7 |
| Dw4 | 3.7 | 3.5 | 4.9 |
| Dw3/Dw4 | - | - | 9.4 |

Table 1.8b

| HLA-DR Specificities | Svejgaard et al. (1980) | Jeannet et al. (1977) | Deschamps et al. (1979) | Serjeantson et al. (1980) |
|---|---|---|---|---|
| DRw3 | 3.7 | 8.07 | 4.0 | - |
| DRw4 | 6.7 | 3.12 | 6.0 | - |
| DRw3/DRw4 | - | - | 46.6 | 37.3 |

(with relative risk values of less than one) with DRw2 in IDDM (Platz *et al*, 1977; Mayr *et al*, 1977).

HLA associations with NIDDM and late onset insulin dependent diabetes

Among Caucasian non-insulin dependent diabetes mellitus (NIDDM) no disturbance in the frequencies of HLA antigens has been observed (Cudworth and Woodrow 1976; Nerup *et al*,1974). Recently, however, two studies have disclosed an HLA antigen association with NIDDM among non-Caucasian populations.

There is a significant increase in frequency of Bw35 and A2 among the IDDM and NIDDM patients of some South African Black populations (Xhosa) unlike the South African white population, relative risk being 2.92 and 2.34 respectively among NIDDM patients (Briggs *et al*, 1980). For the Fiji Indian population, at the HLA-B locus there is a significant increase in Bw61, RR = 3.65 (Serjeantson *et al*, 1980).

Some investigations have attempted to distinguish another type of insulin dependent diabetes which they call late-onset IDDM. Singal and Blajchman (1973) and Cathelineau *et al*, (1975) studied the HLA types in insulin dependent patients in the age range 31-71 years and found an increased relative risk for HLA B8 and Bw15 (2.19 and 2.25 respectively) and a decreased risk for HLA B7 (0.49). No increase in relative risk was found for HLA B18 or for any of the A series antigens.

Ethnic variation in HLA association with IDDM populations

HLA typing among Europeans and North American Caucasians have shown striking increase in A1, B8, B15, B18, Cw3, Dw3 and Dw4 antigens among the IDDM patients. In contrast, other antigens HLA Aw32, B5, B7 and Bw35 and particularly Dw2 are found less commonly in IDDM patients. Studies of the HLA system in non-Caucasian populations have revealed a variety of associations of HLA-B and D locus antigens among IDDM patients.

Among the South African Negro populations, mainly Zulus in Durban, an increased frequency of B8 and B14 are noted (table 1.9). HLA-B7, which is considered a protective antigen among Caucasians, is increased in this population. However, Bw42 detected only in the Black populations, is seen to be lower (Hammond *et al*, 1980). In another study of South African IDDM patients (Hammond and Asmal 1980) Indians who originated in south India showed a positive association with B8 but not with B15, whilst Indians who originated in north India showed an increase of Bw52.

Among Chinese a strong association of DRw3 and DRw4 was noted in IDDM whereas for the B locus antigens there was an increase only of B17 (Maeda *et al*. 1980). Previous results among the Japanese IDDM showed an increase of B12 (Nakao *et al*, 1977). Later, however, a split of Bw22-J (Bw54) was also shown to be increased (Okimoto *et al*, 1978).

Among Mexican American and Black American IDDM similarity with Caucasians is seen in the increased frequency of DRw3 and DRw4, but there is a

complete absence of DRw2 in both populations (Zeidler *et al*, 1980; Rodney *et al*, 1979). As well, DRw3 and DRw4 were increased in USA Black patients: RR 16.7 and 3.9 respectively (Duquesnoy *et al*, 1979).

That the HLA antigens are not themselves the susceptibility genes can be seen from the ethnic distribution of the HLA associations with IDDM, which suggests that susceptibility alleles are in linkage disequilibrium with different HLA alleles in different ethnic groups.

Genetic interpretation of the HLA studies in IDDM and NIDDM

Two lines of explanation emerge from the increased relative risk values among IDDM patients in Caucasians. a. that there is one disease susceptibility gene for IDDM which is in varying disequilibrium with the whole of the HLA system (Cudworth and Woodrow, 1976; Barbosa *et al*, 1977) or in linkage disequilibrium with two or more alleles or HLA (Suciu-Foca *et al*, 1977) and this gene may be recessive with 50% penetrance (Rubinstein *et al*, 1977). b. alternatively, there are two susceptibility alleles within the HLA complex. Supporting evidence comes from the additive relative risk observed in subjects who are both HLA B8 and B15 positive. Since B8 is in linkage disequilibrium with DRw3 and Bw15 with DRw4, association of D locus antigens was observed and, as noted above, RR's are higher for D than for B specificities. A high risk for heterozygotes DRw3/DRw4 (table 1.8b) has been reported among Australian IDDM patients: RR = 37.3 (Serjeantson *et al*, 1980) similar to 46.6 in a French population (Deschamps *et al*, 1979). More detailed study showed Dw4 having an increased risk before age 16, whereas Dw3 increased the risk at all ages supporting the assumption that Dw3 and

Dw4 act by two different mechanisms (Svejgaard *et al*, 1978). If a single recessive gene is in linkage disequilibrium with all other antigens, B8, Bl5, Bl8, Dw3 and Dw4, then the relative risk for B8/Bl5, Dw3/Dw4 heterozygotes should be intermediate to the IDDM risks for B8 or Bl5 and Dw3 or Dw4 alone. But the heterozygotes for B8/Bwl5 and Dw3/Dw4 show a greater relative risk than that of either the B8 or Bl5 and Dw3 or Dw4 by themselves (see tables 1.7 and 1.8b). These increased relative risks form the basis for the hypothesis that two susceptibility alleles are required for clinical IDDM in some cases, refuting the concept of a single autosomal recessive susceptibility allele.

The susceptibility could occur at different HLA-linked loci or at the same locus. If they are at the same locus, inheritance being recessive, then the increased risk of heterozygotes B8/Bwl5 may "be caused by an increased risk for individuals heterozygous for two different 'disease' predisposing alleles compared to those homozygous for one disease allele" (Thomson 1980). Thomson goes on to suggest that a better explanation in keeping with the observed values of the disease incidence in the population is given by assuming that there is one susceptibility allele within the HLA complex and another at a non-HLA locus, since data indicate the possible involvement of more than one locus in predisposing individuals to the disease. The latter allele exhibits no linkage disequilibrium with the HLA disease locus but is relevant in determining an individual's disease susceptibility status (Thomson 1980).

The evidence for heterogeneity within IDDM is based on differential immunologic correlations with different HLA

phenotypes. Using only the information on B8 and B15 but not B18 it is hypothesized (Rotter and Rimoin 1978) that there are three forms of the disease. B8 is associated with an autoimmune form (Form 1). B15 is associated with the insulin antibody responder form (Form 2) and a compound form (Form 3) with both B8 and B15. Hodge *et al*, (1980) have outlined the way in which the three forms could fit with the clinical types of IDDM as outlined in table 1.9.

Table 1.9

Forms of IDDM resulting from different allelic combinations at postulated disease locus (DS) (from Hodge *et al*, 1980)

| DS genotypes | Disease form | Associated with | Characteristics |
|---|---|---|---|
| $S_1S_1$ or $S_1s$ | 1 | B8 | Autoimmunity to the pancreas |
| $S_2S_2$ or $S_2s$ | 2 | B15 | Antibody response to exogenous insulin |
| $S_1S_2$ | 3 | Both | Both of above |
| ss | No disease | - | - |

Mechanisms of action of HLA linked genes

If the immune response genes do exist close to the human HLA locus then linkage disequilibrium may account for specific HLA genes or combinations of them accompanying putative genes responsible for susceptibility to the disease.

Mechanisms are still speculative as to the way in which susceptibility in individuals is conferred. The sources of

evidence suggest two possibilities in the pathogenesis of IDDM. Circulating antibodies (autoantibodies) lead to a suspected autoimmune disease process and, based on the presence of neutralizing antibodies, a viral aetiology is favoured.

In the first case evidence shows that organ-specific anti-pancreatic autoimmunity is a characteristic feature of IDDM (Nerup *et al*, 1977). Insulitis- that is a lymphocytic infiltration of the islets of Langerhans, together with a selective destruction of beta cells is the typical pathological finding in IDDM patients autopsied within the first year (Gepts 1965; Egeberg *et al*, 1976). Circulating islet cell antibodies (ICA) are present in approximately 85% of newly diagnosed diabetic children (Lendrum *et al*, 1976), this feature is characteristic and may aid in distinguishing two types of diabetes. Doniach and Bottazzo (1977) have suggested that, in Type 1A, ICA's are present for a relatively short period whilst, in Type 1B, ICA's are persistent. The latter type is the less common form. The increased duration of IDDM is associated with persistence of islet cell antibodies and the relative risk of having persistent ICA is 2.4 and 3.3 in HLA B8 and B40 patients respectively, increasing in the combined form B8/B40 to 7.2 (Cudworth 1978). In patients with B8/B18, B8/B15 and persistent ICA, relative risk is 4.1 and 2.6. Therefore, a possible pathogenetic mechanism is that the inheritance of 'high risk' alleles produces an appropriate or abnormal immune response to environmental factors (e.g. pancreato-

tropic viruses) compared with subjects who possess genes which may exert a 'protective' effect (e.g. linked with HLA B7) by reducing or modulating the immune response to such agents (Cudworth 1978).

In the second case, however, as reviewed before, evidence of viral infection was seen from the temporal association between outbreaks of viral diseases and onset of IDDM. Further support is provided by the high titres of neutralizing antibodies to Coxsackie B4 virus (Gamble 1976) with increased frequency. Similarly a high prevalence of IDDM has been shown among patients with congenital rubella, predominantly in HLA-B8 positive individuals (Menser *et al*, 1974). However, analysis of mean log antibody titres in relation to HLA phenotypes demonstrated a significant increase in Coxsackie B4 antibody titres in subjects positive for B8/B15 as compared to those positive for either or for B7, and five of the six B8/B15 positive subjects who developed diabetes in 1976 winter peaks had high neutralizing antibody titres to Coxsackie B4 (Cudworth 1978). It is possible that the HLA antigens may act as receptor sites for viral attachment (Svejgaard and Ryder 1977).

Part B

Association of diabetes mellitus with ABO and other blood groups

The suggestion that the ABO blood groups discovered by Landsteiner in 1900, might be involved in the aetiology of a disease, was made as early as 1905 by Dienst (Mourant *et al*,

1978).

For diabetes there is a highly significant overall association with blood group A compared with group O. The total data show a combined relative A/O incidence of 1.07 (Mourant *et al*, 1978). The considerable excess of group A, particularly among men, was significant. This was true for diabetics of "insulin sensitive type" (McConnell *et al*, 1956; Roberts 1957). An increase of blood group A was also noted in Estonian diabetic persons (Ksenofontov 1972). Those cases with onset below 10 years of age show an A/O ratio below unity, which rises throughout life, such that all adult ages show an excess of group A (Andersen and Lauritzen 1960).

In another study by Williams and Cartwright (1979) among the non-insulin dependent diabetics there is an increased frequency of blood group A1 (and A1 + A2), when compared with controls. This difference is particularly marked in male diabetics of the Durham, U.K. population.

Since the occurrence and severity of infections are largely the results of the immunological response of the body to invading micro-organisms it would not be expected if the ABO blood group of an individual were found to be one of the factors determining such a response since A and B blood group substances are commonly found as components of the bacterial cell surface (Mourant 1973). Thus the difference in the frequency of the ABO groups in the patient population, perhaps is due to surface differences in the membrane of the red cells (Dintenfass 1974), or due to

differences in the receptor number and size (Roth *et al*, 1979).

In addition to differences in the ABO blood group frequencies Vague *et al*, (1978) have reported an increased frequency of Lewis-negative blood groups among diabetics in France. However, no association with the Lewis blood groups was found among Durham, U.K. diabetics (Williams and Cartwright 1979). Chakravartti (1967), on the other hand, in a review of blood groups and disease, reported that Lewis-positives were increased in diabetics. All these conflicting results suggest the need for further study of the Lewis blood group system in relation to susceptibility to diabetes.

The reported associations of diabetes with various blood groups opens the possibility of susceptibility alleles being located on some chromosome(s) other than chromosome 6, particularly in the case of NIDDM. As note above, it has been suggested that IDDM and NIDDM may be genetically related (MacDonald, 1974). To explore this possibility further Thomson (1980) suggests that there are two disease loci, $D_1$ with alleles $D_1$ and $d_1$ and $D_2$ with alleles $D_2$ and $d_2$. Susceptibility to NIDDM is determined either solely by $D_2$ (one locus) or by $D_2$ and another locus plus environmental factors, while susceptibility to IDDM is determined by at least two loci, $D_1$ and $D_2$ plus environmental factors. It is further assumed that $D_1$ and HLA are closely linked loci while $D_2$ is unlinked to either. The susceptibility to NIDDM is then conferred by the $D_2$ locus (which in the above instance may be the Lewis-negative blood group) plus

environmental factors.

Chlorpropamide alcohol flushing (CPAF) and diabetes mellitus

CPAF is defined as the occurrence of a facial flush among some individuals after the challenge of 250 mg chlorpropamide followed by 20 mls of dry sherry (Leslie and Pyke 1978).

In NIDDM patients in London with a family background of diabetes, 81% were positive for the CPAF test, whereas only 41% of those without first degree affected family members were CPAF positive. This compares with 1% and 9% in the IDDM and control series. This suggested to one group of workers (Leslie and Pyke 1978) a strong association with NIDDM patients and this was particularly true for the Mason type diabetes, which shows a dominant pattern of inheritance. The relative risk values for CPAF positives given by Leslie and Pyke (1978) are 9 and 38 respectively for individuals without and with a family history of diabetes. On the other hand, in a study of German NIDDM families, no difference in the frequency of the chlorpropamide alcohol flushing was found between those with and without a family history of diabetes (Köbberling *et al*, 1980). The conflict in results from the two sources emphasizes the need for further investigations in order to determine the validity of the CPAF associations.

Scope of the present investigation

Genetic marker studies in relation to disease can use two approaches. The first is that of disease association studies, where the association of the disease with a marker may be statistical in nature and may have an indirect or direct role in the disease process or, alternatively, the subpopulation under study shows an increased frequency of particular alleles at the marker locus, but without a causal relationship.

The second approach is the use of linkage disequilibrium. The close location of genes together on the same chromosome, which travel together without segregation can be used to detect marker gene(s). In such studies the detection of a marker gene linked to genes predisposing to the development of diabetes, clearly could be of great significance in identifying persons at risk.

The present investigation has focussed on both these approaches. In the first approach, data for genetic markers has been collected for NIDDM and control populations from several different ethnic groups in Australia and the Western Pacific. Since the presence of a 'diabetic genotype' has been suggested above it may be expected that there is a significant genetic component in the environmental-genetic interaction contributing to the susceptibility to NIDDM and therefore different patterns of genetic markers may occur in some ethnic groups as compared to others.

The second approach, utilizing the concept of linkage disequilibrium, which is known to occur for the HLA region

on the short arm of chromosome 6, focusses especially on the polymorphic systems such as properdin factor B(Bf), complement factor C´2 of the complement system and the red cell enzyme system Glyoxalase (GLO).

Chapter 2

# POPULATIONS STUDIED AND METHODS

## Sources of samples

Samples from five populations were studied comprising affected and non-affected individuals. They were provided from the sources, outlined below:

| | |
|---|---|
| Australia | Melbourne |
| | Western Australia |
| Pacific Islands | W. Samoa |
| | Nauru |
| India | New Delhi |
| Amerindian (Arizona) | Pima |

## Australia

In Western Australia blood samples were collected as part of a state-wide survey of diabetes sponsored by the Lions Save Sight Foundation and Diabetes Research Foundation. Samples from Kalgoorlie and Geraldton were made available by Dr T. Welborn.

## Melbourne

Samples of patients attending Royal Southern Memorial Hospital, were supplied by Professor P. Zimmet, while those

attending Royal Children's Hospital were supplied by Dr J. Court. In both places diagnosis was made on the basis of clinical criteria supplemented by biochemical information on the level of glucose in the blood, insulin response and presence of ketones, among other parameters. The patients from the Royal Children's Hospital were all dependent on insulin for maintenance and were classified as IDDM. Patients from RSMH were divided into two categories. Those below age 40 at onset of the disease and receiving insulin were classed as IDDM. Those 40 or older at age of onset were classed as NIDDM. Some of these were receiving insulin to maintain them in balance and these are considered separately in the analysis of the data.

Western Australia

Patients examined as part of the diabetes survey were diagnosed by local medical practitioners as diabetic on clinical signs supplemented with abnormal glucose levels. They were divided into categories of IDDM and NIDDM on the basis of age of onset using the criteria outlined for the Melbourne patients. Insulin status also was noted.

Pacific Islands (Western Samoa and Nauru)

Samples collected from Western Samoans and Nauruans were from an epidemiologic survey being carried out in Pacific Islanders under the direction of Professor Zimmet. Diagnosis was done on the basis of 2 hour oral glucose

tolerance test with a glucose load of 75 gm, values above 160 mg glucose/100 ml plasma were considered to be diabetic. Samples were screened from urban and rural areas of W. Samoa. All the patients were described as non-insulin dependent diabetics and treatment was by diet control.

New Delhi

Patients attending the diabetic clinic at the All India Institute of Medical Research, were diagnosed on the clinical criteria. They were classed into categories similar to the Melbourne patients and the samples were kindly supplied by Professor M.M.S. Ahuja.

Pima Indians

Samples were provided by Dr Peter Bennett from his continuing studies of diabetes among Pima Indians living in Arizona. They have been classified as diabetic on the basis of a blood glucose level above 200 mg per cent 2 hours after a 75 mg oral glucose load. Some hyperglycaemic patients were receiving oral anti-diabetic drugs, others were receiving insulin.

## Laboratory Methods

### Handling and transport of samples

The red cell and plasma samples were sent to the laboratory

after the separation of the whole blood. They were packed in dry ice and transported to the laboratory in Canberra. The samples arrived frozen and, in the laboratory, care was taken to thaw the frozen samples for the day's use and store the unused samples in liquid nitrogen.

Laboratory techniques

Discrimination of phenotypes in the haptoglobin, transferrin and ceruloplasmin serum protein systems and all the red cell enzyme systems was made using horizontal starch gel electrophoresis using the technique based on that described by Smithies (1955).

Hydrolyzed starch (Connaught Medical Research Laboratories, Toronto, Canada) was used at approximately 12 per cent (w/v) concentration, modified according to the batch of starch employed. Electrophoresis was carried out between metal cooling plates at approximately 10°C at voltages and times as specificied for the individual system. Banding patterns were developed on the surface of the sliced gels after electrophoresis.

Fifteen polymorphic systems were studied for every population.

Serum Protein systems

| | | |
|---|---|---|
| i | Haptoglobin | Hp |
| ii | Transferrin | Tf |
| iii | Ceruloplasmin | Cp |
| iv | Group Specific Protein | Gc |
| v | Properdin factor B | Bf |
| vi | Complement factor | C`2 |

Red cell enzyme systems

| | | |
|---|---|---|
| vii | Esterase-D | EsT-D |

| | | |
|---|---|---|
| viii | Glyoxalase I | $GLO_1$ |
| ix | Acid Phosphatase | PHs |
| x | Adenylate kinase | AK |
| xi | Adenosine deaminase | ADA |
| xii | Phosphoglucomutase I | $PGM_1$ |
| xiii | Phosphoglucomutase II | $PGM_2$ |
| xiv | Glutamic pyruvic transaminase | GPT |
| xv | 6-Phosphogluconate dehydrogenase | 6PGD |

Serum protein systems

For haptoglobin, transferrin and ceruloplasmin, Ashton and Bradon (1961) discontinuous buffer system was used. The plasma samples, separated from the red cells were thawed and inserted into the gels on strips of Whatman No. 3 filter paper.

Electrode buffer

| Solution A | gm/l |
|---|---|
| Lithium hydroxide | 1.2 gm |
| Boric acid | 11.8 |

Gel buffer

Solution B

| | |
|---|---|
| Citric acid | 1.6 gm |
| Tris | 6.2 gm |
| pH = 8.0 | |

The solution B was diluted 9B : 1A for the preparation of gels. Voltage reading on the meter was 400 v., the gels were electrophoresed for 4 hours.

Staining

Gels were sliced for staining of ceruloplasmin and haptoglobin on one half using the following reagents; 2mg.0-dianisidine dissolved in 1 litre distilled water with 5 ml acetic acid. It was mixed and filtered. The gels were incubated at 36°C solution for ½ hour; faint orange bands of ceruloplasmin appear. 1 ml

per gel of hydrogen peroxide was then added and the dark green bands of Hp-Hb complex appear below the ceruloplasmin zone, after a further 10-15 minutes. On the other half of the same gel, transferrin was visualized using amido black stain: 2 gms of the dye dissolved in a methanol-water-acetic acid solution in proportions 50-50-10 respectively. Staining requires only a few minutes, after which the gel is decolorized in the same solution without the dye. The transferrin band is clearly recognized behind the free haemoglobin protein band.

Properdin factor B and Group Specific Component

Immunoelectrophoretic methods were used for identifying Properdin factor B and the Group Specific Component (Gc). This involves the initial electrophoretic separation of the serum protein components followed by immunofixation against anti-human properdin factor B serum and anti-human Gc globulin serum. These were obtained from Atlantic Antibodies, U.S.A.: Anti: GBG Grade N (goat) and Anti: Gc globulin Grade SP (goat).

Electrophoresis was carried out using Agarose gel as a medium (conc 1%) on glass plates 6 x 6 3/4". Buffer system used for Gc and Bf was that given by Johnson *et al* (1975). The quantity of plasma used is 2-3 µls per sample.

| Stock buffer | gm/litre |
|---|---|
| Barbitone (barbituric acid) dissolved in hot water | 2.5 |
| Sodium Barbital | 15.4 |
| Calcium lactate | 0.616 |

The gel buffer was 2 parts stock buffer and 1 part distilled water. Stock buffer was used in the electrode vessels. A voltage gradient of 13 volts/cm and electrophoresis for 1 to 1.5 hours was conducted for Gc, after which anti-Gc sera was layered and incubated for 1 hour at room temperature. Staining was carried out by using the coomassie blue dye, dissolved in 4.5:1.4.5 proportion of methanol-acetic acid-water. The quantity of antisera was determined at 0.2 mls diluted with 0.8 mls saline per gel. The same procedure was followed for Bf, the difference being:

a) electrophoresis for longer time - 4 hours, voltage gradient (13 v/cm).
b) use of anti-Bf antisera.
c) concentration of antisera 0.2 ml diluted in 1.5 ml saline per gel.

Complement component C'2

The methods widely used for the separation of complement components have been by gel electrophoresis, usually in agarose and isoelectrofocussing in polyacrylamide gels. In the case of C'2 complement component isoelectric focussing technique was used, as described by Lachmann and Hobart (1978). The solutions used were:

| Acrylamide solution | gm/500 mls |
|---|---|
| Acrylamide | 33.5 |
| nn' methylenebis acrylamide | 0.9 |
| Taurin (dissolved in distilled water) | 16.68 |
| To the above is added: | |
| Ampholyte | 1.5 mls/gel (Pharmacia) pH range 4-6.5 |

| | |
|---|---|
| Temed | 15 µl |
| Riboflavin | 7 mls |

The mixture is degassed and poured into a 1 mm thick glass mould and left under a fluorescent tube light source until polymerization has taken place.

Samples

Serum samples 20 µl, in good condition are applied on Whatman 3 mm paper strips placed 1" from one end of the gel.

Isoelectrofocussing

This is done by placing the loaded gels with samples, face downwards across the carbon-rod electrodes of focussing box with the gel in continuous contact with the electrode and the samples to the inside of the anode. Voltage is set to a maximum of 1000 v. with a current less than 10 mA/gel, and electrophoresis carried out for 18 hours, overnight. The bands of C´2 complement activity were revealed using a haemolytic assay in an agarose overlay. Before the application of gels overlay, the gels are soaked in an iodine solution for 30 minutes at room temperature, made as follows:

| Stock solution | gm/100 mls |
|---|---|
| Iodine | 0.25 |
| Potassium iodide | 8.3 |
| dissolved in phosphate Buffer 0.5 M | 40 mls, made up to 100 mls with distilled water. |
| pH = 6.0 | |

Iodine solution

| | |
|---|---|
| Iodine stock solution | 25 mls |
| NaCl | 4.5 gm made up to 500 mls with distilled water |

The gels are later soaked in complement fixation diluent (CFD) tablets from Oxoid Ltd., London, for 30 minutes at room temperature. Perspex strips are placed around the area of the gel where bands are expected and are sealed in position with agarose. The overlay for the staining procedure was made using the following reagents:

Phosphate buffered saline (PBS)

2% by volume of 0.2M phosphate buffer (pH 7.2) in 0.85% NaCl.

EDTA-Saline

0.01M EDTA in PBS prepared by adding 5% by volume of 0.2M EDTA (pH 7.2) to PBS.

Sensitized sheep red cells

Sheep red cells were collected in Alsevers solution, spun to separate cells from plasma, washed three times in PBS and suspended in 10% PBS solution. Since the complement system is a triggered enzyme system of the blood plasma, its action is usually initiated by the combination of antibody and antigen. The sheep red blood cells are coated with antibody, then known as EA.

For 1 ml EA :

1 ml 10% E (erythrocytes)
1 ml EDTA - saline
10 µl rabbit antisheep antibody (Wellcome Rabbit haemolytic serum)

Antibody is added to EDTA solution and mixed with 10% E at 4°C for 10 mins. This mixture is spun and washed once in EDTA - saline and twice in CFD and reconstituted to 1 ml with CFD.

C´2 deficient serum

This was prepared by heating selected normal human serum to 50°C for 20 minutes.

Gel overlay

7.5 mls of CFD for each gel is heated in a water bath (about 50°C), 7.5 mls of 2% agarose solution is added. After removing from water bath 0.75 mls of 10% sensitized sheep red cells and 0.2 ml C´2 deficient human serum is added. This is mixed well and poured over the gel spreading evenly. The overlay is allowed to set before removing the perspex strips. The gels are then placed in an airtight box containing a moist sponge and incubated at 4°C for 1 hour and then at 37°C until bands of lysis appear.

## Red cell enzyme systems

As indicated above all red cell enzyme systems were typed using horizontal starch gel electrophoresis techniques, with buffer systems and substrate procedures appropriate to each system. In all cases haemolysates prepared from frozen and thawed packed red cells were inserted into the gels on strips of Whatman No. 3 filter paper, except for GPT where No. 17 paper was employed. With the exception of PHs and 6PGD enzymes

hemolysates, the other systems were first treated before inserting in the gel, incubating with 0.01 ml 1% 2-mercaptoethanol per ml of haemolysates for 30 mins at room temperature to improve the sharpness of the bands.

Esterase-D and Phosphoglucomutase (locus 1 and 2)

| Electrode buffer | gm/litre |
|---|---|
| Tris | 12.1 |
| Maleic acid | 11.6 |
| EDTA Na2 | 3.7 |
| Magnesium sulphate | 2.4 |

Adjusted to pH 7.4 with NaOH (approximately 4.5 gm/L).

Gel buffer

Electrode buffer diluted 1:15 for $PGM_1$ and $PGM_2$

Voltage used : 170v. on meter.

Staining

The gels are sliced and the top half used for staining EsT-D with 2.5 mg of 4-methylumbelliferyl acetate dissolved in a small quantity of acetone in acetate buffer (diluted 1 in 10) pH 5.2 (Spencer *et al*, 1964). Filter paper is soaked in 10 mls of the above solution and placed on the gels for 10 minutes after which they are read under u.v. light. The bottom half is stained for $PGM_1$ and $PGM_2$ using a reaction mixture composed of:

| | mg/gel |
|---|---|
| Glucose-1-phosphate | 30 |
| Magnesium sulphate | 5 |
| Nicotinamide adenine dinucleotide phosphate (NADP) | 7 |
| Phenazine methosulphate (PMS) | 5 |
| (3-(4,5-di-methyl-diphenyl teterzolium bromide MTT) | 5 |
| Glucose-6-phosphodehydrogenase (G6PD) | 4µl |

Dissolved in 10 ml 0.1M Tris-hydrochloric acid buffer pH 8.2 to which is added 10 ml of 1% (w/v) agar at approximately 60°C. This is overlayed on the gel and incubated for 10-15 minutes at 36°C. $PGM_1$ and $PGM_2$ appear dark blue.

Glyoxalase I

| | gm/litre |
|---|---|
| Electrode buffer | |
| Tris | 12.1 |
| Citric acid | 7.1 |
| Boric acid | 2.4 |
| Lithium hydroxide | 1.0 |
| pH = 7.2 | |

Gel buffer

Electrode buffer diluted 1 in 10. Voltage used 80v.

Staining

This is a two step procedure, Kompf *et al* (1975b)

First step - filter paper overlay

| | |
|---|---|
| Methylgloyoxal (pyruvic aldehyde) | 0.23 mls/gel |
| Glutathione | 7.5 mg/gel |

This is dissolved in a buffer mixture of 2.75 ml/gel monobasic phosphate buffer and 2.25 ml dibasic phosphate buffer of pH 6.8. The gels are incubated for 30 minutes.

Second step - Agar overlay

18 mg of MTT dissolved in 0.1M Tris-hydrochloric acid buffer, 15 mls/gel pH 8.5. To this was added approximately 4ml dichloro-indophenol, a blue dye, in the same quantity of agar as the buffer. The gels were incubated for 10-15 minutes at 36°C. Glyoxalase I bands appeared light green and the three phenotypes were discriminated according to Kompf *et al*, (1975b).

Acid phosphatase

| Electrode buffer | gm/litre |
|---|---|
| Sodium dihydrogen phosphate | 38.2 |
| Tri-Na-citrate | 44.1 |
| di-Sodium EDTA | 1.3 |
| pH = 6.0 | |

Gel buffer

Electrode buffer diluted 1 in 100. Voltage used 150v.

Staining

Procedure is a simple filter paper overlay, soaked in 2 mg of 4-methylumbelliferyl phosphate in 10 mls of 0.05M citrate buffer and incubated for 15-20 minutes (Karp and Sutton 1967). To discriminate the phenotype $PHs^C$ from $PHs^A$ and $PHs^B$ the alternative method by Hopkinson *et al*, (1964) was used. The substrate consists of:

| | |
|---|---|
| Phenolphthalein diphosphate | 143 mg |
| Citric acid | 10.5 gm/l |
| Sodium hydroxide | 5.3 gm/l |

dissolved in 50 ml of 0.05M citrate buffer, pH 6.0. The gels are incubated in this solution for 3-4 hours. 2 mls of concentrated ammonium hydroxide is then added, when pink coloured strong bands are visible.

Adenylate kinase and adenosine deaminase

Electrode buffer

| | gm/l |
|---|---|
| Solution A: | |
| Potassium dihydrogen phosphate | 68.0 |
| Solution B: | |
| Disodium hydrogen phosphate | 70.9 |

The two solutions in proportion 1 : 2 i.e. 38 mls soln. B mixed with 76 mls soln. A, are made up to pH 6.5 (Fildes and Harris 1966).

Gel buffer

Electrode buffer diluted 1 in 10 mls. Voltage used 100v.

Staining

| AK | mg/gel |
|---|---|
| Glucose | 40 |
| Magnesium chloride | 200 |
| Adenosine -5' diphosphate (ADP) | 25 |
| Nicotinamide adenine dinucleotide phosphate (NADP) | 15 |
| Phenazine methosulphate (PMS) | 5 |
| MTT | 5 |

Dissolved in 10 ml (per gel) of Tris/hydrochloric acid buffer pH 8.2. Enzymes G6PD - 0.4 µl and hexokinase 0.08 µl are added to the solution. Equal quantity of 1% agar is mixed and poured over the gel. Dark blue bands appear close to the origin.

| ADA | mg/l |
|---|---|
| Adenosine | 9 |
| MTT | 2 |
| PMS | 2 |
| Nucleoside phosphorylase (NP) | 20 µl |
| Xanthine oxidase (XOD) | 40 µl |

Dissolved in 10 ml/gel Tris-hydrochloric acid buffer pH 8.5,

with equal quantity of agar and poured over the gel. Dark bands appear at the centre of the gel.

Glutamic pyruvic transaminase

| Electrode buffer | gm/l |
|---|---|
| Tris | 12.1 |
| Citric acid | 6.1 |
| pH = 7.5 | |

Gel buffer

Prepared by diluting electrode buffer 1 in 10 ml distilled water. Voltage used 240v.

Staining

| | quantity for 6 gels |
|---|---|
| Alanine solution (100mg/ml in tris 0.1M HCL pH 7.6) | 7.2 mls. |
| a-ketoglutarate soln.(10mg/ml in tris 0.1M HCL pH 7.6) | 7.8 mls. |
| Nicotinamide adenine dinucleotide hydrogen (NADH) | 40 mg |
| Lactate dehydrogenase | 100 µl |

Dissolved in 25 ml Tris/HCL pH 7.6, soaked on to No. 1 filter paper and incubated for 3-5 hours and observed under U.V. light (Chen and Giblett 1971).

6-Phosphogluconate dehydrogenase

| Electrode buffer | mg/l |
|---|---|
| Di-sodium hydrogen phosphate (anhydrous) | 28 |
| Citric acid | 4.6 |
| pH = 7.0 | |

Gel buffer

Electrode buffer dilute 1 in 20 mls (Fildes and Parr 1963)

voltage used 80v.

| Staining | mg/gel |
|---|---|
| NADP | 2 |
| 6-Phosphogluconic acid | 10 |
| PMS | 0.4 |
| MTT | 2 |

Dissolved in 10 mls Tris/HCL buffer pH 8.2 and equal quantity of agar is added. Incubation period of 10 minutes shows blue bands at the anodal end.

## Statistical methods

### Gene frequencies

The loci studied in the present investigation are all controlled by autosomal codominant alleles. Gene frequencies therefore were estimated directly be gene counting. Goodness-of-fit to random mating proportions, assuming Hardy-Weinberg equilibrium was tested by the appropriate chi-square test (Cavalli-Sforza 1971).

### Test for association

The data were categorized into contingency tables for two populations at a time (controls vs. patients) and association examined by chi-square tests for heterogeneity. Whenever a significant $\chi^2$ appeared the situation was examined more closely to identify where the heterogeneity lay and to see whether similar trends occurred in the different sexes and different age groups. This typically involved the grouping together of

some phenotypes. The method of Woolf (1955) advocated for associations analysis is most suitable for large sample sizes, but it was applied, in some cases, to confirm the significant results.

Distance/multivariate analysis

Analysis of heterogeneity within diabetes mellitus, represented by various affected populations, was examined also using statistical program of genetic distance analysis between populations. In this case the disease groups were subjected to such an analysis using a program for Morton's Biokin distance provided by the Department of Human Biology. This procedure essentially follows Wahlund's (Morton 1970) approach and compares gene frequencies of subdivisions of the population with the population mean (Friedlænder 1974). The computer program, Topol Arbor, was used to provide the dendrograms and two dimensional eigenvectorial representation for the population distance.

Chapter 3

# AUSTRALIAN POPULATIONS

## Section 1 : Melbourne

The discussion for this chapter will be considered in two sections. Section 1 discusses the series of diabetic patients from Melbourne and Section 2, those from Kalgoorlie and Geraldton in Western Australia (See chapter 2 for details of the series). For comparison with the patients, non-affected persons as controls were derived from blood donors in Canberra and Sydney.

- those on insulin treatment and below age 40 years (IDDM)
- those not on insulin but treated by a controlled diet and oral agents, with age 40 and above (NIDDM)
- a third category, described for the present study alone, are those 40 and above but receiving insulin therapy currently. These are categorized below as Mature insulin dependent diabetes mellitus (MIDDM).

The observations have been made with particular reference firstly to non-HLA chromosome 6 marker genes as described earlier: glyoxalase, properdin factor B and complement factor C´2. The studies of markers on other chromosomes will be discussed later.

Note: Due to the inability to type some samples for the serum proteins and red cell enzyme systems, the totals shown in the tables are, therefore, not all the same. This applies to all populations discussed in the subsequent chapters.

### Chromosome 6 markers

#### Glyoxalase I (GLO)

The results obtained are shown in table 3.1.1. In the

Table 3.1.1

Distribution of glyoxalase (GLO) phenotypes and gene frequencies in the total diabetic and control series population of Melbourne

| Population | Phenotypes | | | No. tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $GLO^1$ | $GLO^2$ |
| Control | 72 | 195 | 115 | 382 | 0.4437 | 0.5563 |
| IDDM | 46 | 128 | 75 | 249 | 0.4418 | 0.5582 |
| NIDDM | 44 | 97 | 88 | 229 | 0.4040 | 0.5960 |
| MIDDM | 17 | 33 | 31 | 81 | 0.4136 | 0.5864 |

Test for heterogeneity of phenotypic distribution NIDDM vs. control: $\chi^2_2 = 5.30$ $P<0.05$

MIDDM vs. control: $\chi^2_2 = 2.5$ $P<0.2$

IDDM vs. control: $\chi^2_2 = 0.01$ n.s.

When combined NIDDM+MIDDM vs. control: $\chi^2_2 = 6.56$ $P<0.02$

Gene frequency NIDDM vs. control: $\chi^2_1 = 1.89$ $P>0.25$

NIDDM+MIDDM vs. control: $\chi^2_1 = 1.44$ $P<0.20$

Note

The control data compatible with random mating (H.W. equilibrium) $\chi^2_1 = 0.32$ while the combined classes of diabetics NIDDM+MIDDM deviate from this hypothesis $\chi^2_1 = 4.78$ $(0.05<P<0.02)$.

NIDDM and MIDDM series, the phenotypic distributions show an 8-10% decrease in the heterozygotes GLO 2-1, with a corresponding increase in the homozygotes GLO 2-2 as compared with controls and IDDM series. When tested for heterogeneity for the distribution of phenotypes in NIDDM versus control series, the chi-square value for 2 degrees of freedom obtained was 5.30 ($P<0.05$). For the MIDDM series versus controls $\chi^2_2 = 2.5$ ($P<0.2$). When the above two series are combined into a single series of maturity onset diabetes and tested for heterogeneity $\chi^2_2 = 6.56$ ($P<0.02$).

Part of this heterogeneity may be due to ethnic stratification of the patient population compared with that of the controls. The disturbance shows an increase of $\underline{GLO}^2$ gene frequency and also a relative deficiency of 2-1 heterozygotes which is reflected in the Hardy-Weinberg test for random mating and which, in the case of NIDDM of the present series, gives a $\chi^2_1 = 4.8$ ($0.05>P>0.02$).

To check if ethnic stratification is playing a significant role in giving these results, the patients and controls were classified on the basis of family name or by response to questions, into groups by region of origin in Europe. People of Southern and East European and Middle East origin constituted approximately 25% of the total. The remainder were North European (mainly British) in origin. Table 3.1.2 gives the distribution of GLO phenotypes for patients and controls of north European origin only. Heterogeneity between the two series has been greatly decreased and there is no longer any significant difference ($\chi^2_2 = 3.8$: $0.2>P>0.1$).

Table 3.1.2

Distribution of glyoxalase phenotypes and gene frequencies in the North European diabetic and control series population of Melbourne

| Series | Phenotypes | | | Gene frequency | |
|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | $\underline{GLO}^1$ | $\underline{GLO}^2$ |
| Control | 31 | 88 | 54 | 0.4370 | 0.5630 |
| NIDDM + MIDDM | 51 | 101 | 91 | 0.4177 | 0.5823 |

Test for heterogeneity of phenotypes in patients vs. control series $\chi^2_2 = 3.80$ $P<0.1$

Table 3.1.3

Distribution of glyoxalase phenotypes and gene frequencies in the diabetic population of Poles, Russian and Czechoslavakian in the Melbourne series

| Series | Phenotypes | | | Gene frequency | |
|---|---|---|---|---|---|
| n = 45 | 1-1 | 2-1 | 2-2 | $\underline{GLO}^1$ | $\underline{GLO}^2$ |
| NIDDM + MIDDM | 6 | 18 | 21 | 0.3333 | 0.6667 |

Among the Eastern European (Poles, Russian and Czech) population, there were 45 people of Polish origin and the $\underline{GLO}^2$ allele had a high frequency of 0.6667 among them (table 3.1.3) contributing to the excess of the GLO 2-2 phenotypes in the total series. Since the Southern Memorial Hospital is located in an area with a large population of East European origin, which could result in the excess of those members attending the hospital and therefore contributing to the distortion in the GLO phenotypes observed in the diabetic patients.

Complement factor : Properdin factor B(Bf)

As seen from table 3.1.4, from a total of 242 IDDM patients, the number of persons with the Bf $F_1$ factor was 28 as compared to 8 in the control series. The frequency of the two rare alleles $\underline{Bf}^{F1}$ and $\underline{Bf}^{S1}$ were increased significantly when compared to controls. $\underline{Bf}^{F1}$ is present in 1.0% of the controls, whereas it is 6.0% in the IDDM series and 0.40% and 0.0 among the NIDDM and MIDDM patients. $\underline{Bf}^{S1}$ shows an increase of 0.9% in the IDDM when compared to controls. It is 1.1% in NIDDM and 0.6% in the MIDDM series. Gene frequency for the $\underline{Bf}^{S}$ allele shows an increase of 3% in the IDDM in the present series: $\underline{Bf}^{S}$ = 0.8285 compared with $\underline{Bf}^{S}$ = 0.7906 in controls. This does not correspond with the increase of 16.2% shown by Cudworth *et al* (1977) among their IDDMs below 30 years. However, there is a sharp decrease in the $\underline{Bf}^{F}$ allele frequency in this IDDM series to $\underline{Bf}^{F}$ = 0.0971 from 0.1937 in controls.

When subjected to analysis of heterogeneity of gene frequencies in patients versus control series (table 3.1.4a), the increase

Table 3.1.4

Distribution of Properdin factor B(Bf) phenotypes and gene frequency in the diabetic and control population of Melbourne

| | Phenotypes | | | | | | | | No. tested | Gene frequency | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SS | FS | $SS_1$ | FF | $FS_1$ | $SF_1$ | $FF_1$ | $F_1F_1$ | | $\underline{Bf}^S$ | $\underline{Bf}^F$ | $\underline{Bf}^{F1}$ | $\underline{Bf}^{S1}$ |
| Controls | 248 | 100 | 3 | 20 | 1 | 5 | 3 | 0 | 380 | 0.7906 | 0.1937 | 0.0105 | 0.0052 |
| IDDM | 166 | 38 | 6 | 3 | 1 | 25 | 2 | 1 | 242 | 0.8285 | 0.0971 | 0.0599 | 0.0145 |
| NIDDM | 160 | 54 | 4 | 8 | 1 | 2 | 0 | 0 | 229 | 0.8297 | 0.1550 | 0.0044 | 0.0109 |
| MIDDM | 54 | 25 | 0 | 1 | 1 | 0 | 0 | 0 | 81 | 0.8210 | 0.1728 | 0.0000 | 0.0062 |

Table 3.1.4b.

| | S | F | $S_1$ | $F_1$ |
|---|---|---|---|---|
| Control | 79.5 | 19.3 | 0.5 | 1.0 |
| IDDM | 82.8 | 9.7 | 1.5 | 6.0 |

Tests for heterogeneity of per cent gene frequencies in IDDM vs. controls.

$\underline{Bf}^{F1} + \underline{Bf}^{S1}$ in IDDM vs. controls $= \chi^2_1 = 22.8$ $P<<0.001$

$\underline{Bf}^{F} = \chi^2_1 = 21.5$ $P<<0.001$

Similarly, for heterogeneity of percent gene frequency in NIDDM vs. controls.

$\underline{Bf}^{F} = \chi^2_1 = 2.1$ $P<0.10$

Tests for heterogeneity of per cent gene frequencies when only North European population is considered.

$\underline{Bf}^{F1} + \underline{Bf}^{S1}$ in IDDM vs. controls $= \chi^2_1 = 18.5$ $P<<0.001$

$\underline{Bf}^{F} = \chi^2_1 = 16.4$ $P<<0.001$

Association test for $BfF_1 + S_1$ by Woolf's $\chi^2_1 = 20.8$ $P>0.001$

of the rare alleles $\underline{Bf}^{S1}$ and $\underline{Bf}^{F1}$ in IDDM patients was highly significant ($\chi^2_1$ = 22.8 P<<0.001). Further analysis of the North European population alone showed the combined frequency of $\underline{Bf}^{F1}$ and $\underline{Bf}^{S1}$ also to be significantly increased over controls ($\chi^2_1$ = 18.5 P<0.001). The $\underline{Bf}^{F}$ frequency also was decreased significantly ($\chi^2_1$ = 16.4 P<0.001). These results demonstrate a strong association of Bf factors with IDDM in the Melbourne IDDM patients.

No significant differences in the occurrence of rare alleles were observed in the NIDDM and MIDDM series when compared to controls.

The individuals with the Bf $F_1$ factor can be said to be at a relatively greater risk than those without. The relative risk value calculated for the Australian IDDM patients with Bf $F_1$ factor is 6.2 and when combined with Bf $S_1$, RR = 5.3. This value is much lower than that shown by Raum *et al*, 1979 on the U.S. white population (table 3.1.5) where RR for Bf $F_1$ = 15.0. Other workers have shown, for the Newfoundland population of Canada, a relative risk for Bf $F_1$ = 5.3 (Dornan *et al*, 1980) and, in the German population, RR = 3.4 (Bertrams *et al*, 1979; Mouzon *et al*, 1979) also showed an increase of Bf $F_1$ in the French Basque children with a relative risk value of 11.4 for those with the Bf $F_1$ factor.

Table 3.1.5

Relative risk values of Bf $F_1$ reported in different populations.

| Reference | R.R. |
|---|---|
| Raum *et al*, 1979 | 15.0 |
| Bertrams *et al*, 1979 | 3.37 |
| Dornan *et al*, 1980 | 5.55 |
| Present study | 6.20 |

The discrepancy in the estimates of relative risks between the U.S. and Australian IDDM series for the Bf $F_1$ factor, may well reflect a different age composition in the two studies.

Age relationship between insulin-dependent diabetes and rare alleles of Properdin factor B.

Since the onset of IDDM is related to age, a further breakdown into groups based on age of onset, was carried out. It may be noted that the results published earlier (Kirk *et al*, 1979), differ from those described below in the use of the age criteria, although the trend of distribution for Bf $F_1$ remains the same. Since more detailed information was available on the IDDM individuals, accurate analysis was performed based on the age of onset of the individuals.

The age groups included a range of 0-4; 5-9 and 10-14 years. Distribution of the Bf $F_1$ factor in these age groups is shown in figure 3.1. The frequency of Bf $F_1$ in the age groups 0-4, is 18.5% and in the 5-9 group, 13.6%, falling to 11.1% in the 10-14 age group, in comparison to the controls with a frequency of 3.2%. However, for the other factor, Bf $S_1$, although a progressive decline with age is not observed, interestingly in the 0-4 and 5-9 age groups,

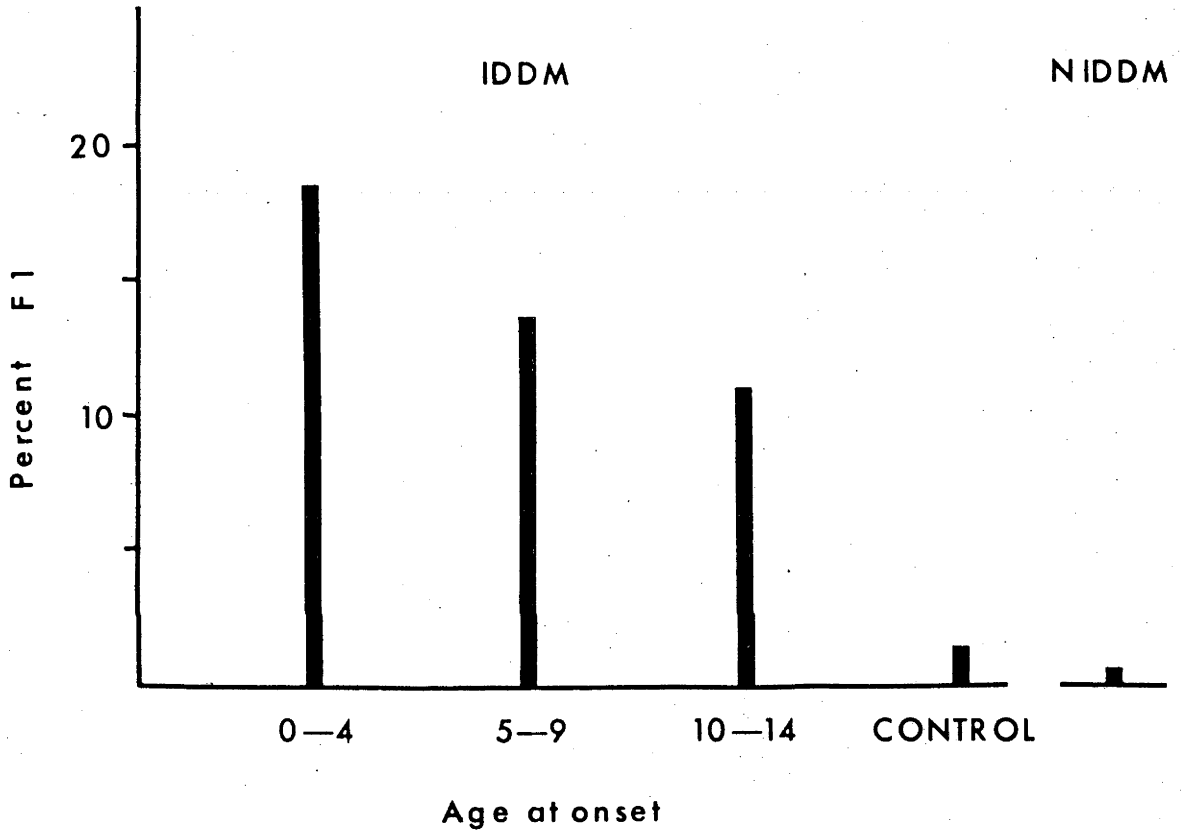



Fig. 3.1. Relationship of age of onset to frequency of rare Bf factors in Melbourne IDDM patients.

the Bf $S_1$ frequencies are 3.7% and 7.5% respectively, falling sharply in the 10-14 and over age groups. The relative risk for Bf $F_1$ and Bf $S_1$ combined is 9.4 in patients below 10 years. The rapid decline in the frequency of Bf $F_1$ with increasing age of patients with IDDM is compatible with a viral stimulus for initiating the disease process. Implications of this consideration will be discussed in greater detail in the last chapter. Such an age-based correlation in IDDM has been reported previously with HLA-B15 (Dausset 1977).

Analysis of HLA and Bf factors.

Since the Bf locus resides within the HLA complex, studies for the assocation of HLA antigens with Bf factors were carried out. This analysis was made possible by using the results of HLA typing carried out by Mr. Philip Kiely in Melbourne. Only the HLA-A and B antigens were typed and, due to the non-availability of the serum,HLA-B18 was not included in the array of antigens tested.

The percentage of HLA-B antigens present in the IDDM and control series is shown in table 3.1.6. The comparison reveals a significant increase of B8 (48% vs.24%) and B15 (21% vs. 8%). There is a significant decrease of Bw35 (3% vs. 16%).

Table 3.1.6

Per cent distribution of HLA-B antigens in Melbourne IDDM patients and controls (from Kiely *et al*, 1978)

| HLA-B | IDDM | CONTROL |
|---|---|---|
| B7 | 17 | 21 |
| B8 | 48 | 24 |
| B12 | 21 | 25 |
| B15 | 21 | 8 |
| B21 | 4 | 1 |
| B35 | 3 | 16 |

Pairwise associations were tested for significance by 2 x 2 contingency tables using Fisher's $\chi^2$ test of significance. Examples of these contingency tables are given in tables 3.1.7A, B and C. The derived probability values for these pairwise comparisons are summarized in table 3.1.7. Significant positive disequilibrium at probabilities of P = 0.05 or lower in the case of IDDM patients, was demonstrated for HLA-A1 with Bf F: significant negative disequilibrium values were obtained for HLA-B8 with Bf F, $F_1$ and $S_1$ and also for HLA-Bw35 and BfF.

Table 3.1.7A

2 x 2 contingency tables for HLA antigens and Bf factors in IDDM patients and controls

IDDM patients

| S \ B8 | - | + |
|---|---|---|
| - | 5 | 1 |
| + | 78 | 77 |

$\chi^2_1 = 1.371$

P = 0.241

Controls

| S \ B8 | - | + |
|---|---|---|
| - | 21 | 43 |
| + | 0 | 3 |

$\chi^2_1 = 0.144$

P = 0.750

| F \ B8 | - | + |
|---|---|---|
| - | 62 | 73 |
| + | 21 | 5 |

$\chi^2_1 = 9.248$

P = 0.002

| F \ B8 | - | + |
|---|---|---|
| - | 25 | 16 |
| + | 21 | 5 |

$\chi^2_1 = 2.897$

P = 0.100

NOTE: That BfF factor is in significant negative disequilibrium with HLA-B8 antigens in IDDM

Table 3.1.7B

2 x 2 contingency tables for HLA antigens and Bf factors in IDDM patients and controls

IDDM patients

| $S_1$ | B8 − | B8 + |
|---|---|---|
| − | 77 | 78 |
| + | 6 | 0 |

$\chi^2_1 = 4.015$

$P = 0.045$

exact $P = 0.034$

Controls

| $S_1$ | B8 − | B8 + |
|---|---|---|
| − | 45 | 20 |
| + | 1 | 1 |

$\chi^2_1 = 0.360$

$P = 0.500$

| $F_1$ | B8 − | B8 + |
|---|---|---|
| − | 68 | 73 |
| + | 15 | 5 |

$\chi^2_1 = 4.012$

$P = 0.045$

| $F_1$ | B8 − | B8 + |
|---|---|---|
| − | 39 | 21 |
| + | 7 | 0 |

$\chi^2_1 = 3.568$

$0.10 < P < 0.05$

exact $P = 0.123$

NOTE: That the $BfF_1$ and $S_1$ factors in IDDM patients show significant negative disequilibrium with HLA-B8 antigen as compared to controls.

Table 3.1.7C

2 x 2 contingency tables for HLA antigens and Bf factors in IDDM patients and controls

IDDM patients

| F \ Bw35 | - | + |
|---|---|---|
| - | 134 | 1 |
| + | 22 | 4 |

$\chi^2_1$ = 11.050

P = 0.0009

Controls

| F \ Bw35 | - | + |
|---|---|---|
| - | 35 | 6 |
| + | 25 | 1 |

$\chi^2_1$ = 1.979

P = 0.250

| F \ Al | - | + |
|---|---|---|
| - | 72 | 63 |
| + | 20 | 6 |

$\chi^2_1$ = 4.037

P = 0.044

| F \ Al | - | + |
|---|---|---|
| - | 29 | 12 |
| + | 22 | 4 |

$\chi^2_1$ = 1.680

P = 0.250

NOTE: A highly significant negative disequilibrium in the patient series occurs between HLA-Bw35 and BfF factor.

Table 3.1.7.

Selected HLA antigens and Bf factors: 2 x 2 probability values for IDDM patients and controls

| HLA | BF S | $S_1$ | F | $F_1$ | S | $S_1$ | F | $F_1$ |
|---|---|---|---|---|---|---|---|---|
| $A_1$ | 0.367 | 0.081 | 0.045 | 0.138 | - | - | 0.025 | - |
| B8 | 0.242 | 0.045 | 0.002 | 0.045 | 0.750 | 0.500 | 0.100 | 0.050 |
| B15 | 0.518 | 0.669 | 0.463 | 0.788 | - | - | - | - |
| B35 | 0.451 | 0.451 | 0.0009 | 0.868 | - | - | 0.250 | - |

As expected, the positive association of HLA antigens with a Bf factor is almost always paralleled by a negative correlation with the other Bf factor (Albert *et al*, 1977). For this reason the negative disequilibrium between HLA-B8 and Bf F probably reflects the known strong positive disequilibrium between B8 and Bf S in the normal European population (Albert *et al*, 1977; Arnason *et al*, 1977).

The negative disequilibrium value between HLA-B8 and Bf $S_1$ and Bf $F_1$, both of which have significantly increased frequency in the present investigation of Melbourne IDDMs, suggests that there is a diabetic susceptibility allele in linkage disequilibrium with HLA-B8 in some cases and with $\underline{Bf}^{F1}$ and $\underline{Bf}^{S1}$ in others.

## Family Investigation

In the introductory chapter it was pointed out that the HLA polymorphism has provided a powerful tool in genetic studies of

IDDM, providing possible clues to its aetiology. Using the information of HLA association and linkage on chromosome 6, several investigations of the mode of inheritance were attempted by noting the percentage of pairs of diabetic siblings who share the same HLA haplotype in families with more than one child affected. The frequency of pairs of diabetic sibs with identical haplotypes was 50-60%, much higher than the 25% expected by chance (Cudworth and Woodrow,1975; Barbosa *et al*, 1977;Rubinstein *et al*, 1977; Spielman *et al*, 1978). This suggests the close linkage of the IDDM susceptibility allele within the MHC complex.

In the present study, segregation at another linked locus (Bf) within the HLA complex, is examined. Segregation of the Bf rare alleles independently and in linkage with other HLA alleles is considered, although very few families are informative for the rare alleles.

Thirteen multiple case families were available from the Royal Children's Hospital, Melbourne and fourteen from the Newcastle area of N.S.W. (kindly made available by Dr.H. Bashir). These families showed either an affected parent and child or only affected children. In the former series three families were informative for the Bf rare alleles and five of the latter series. Combining the total of eight informative families, four had either parent and one child affected or both sibs affected and, in the remaining four, only one child was affected.

Number of IDDM affected + Bf $F_1$ = 8

Number of unaffected + Bf $F_1$ = 10

Both normal and affected persons are almost equally positive

for Bf $F_1$.

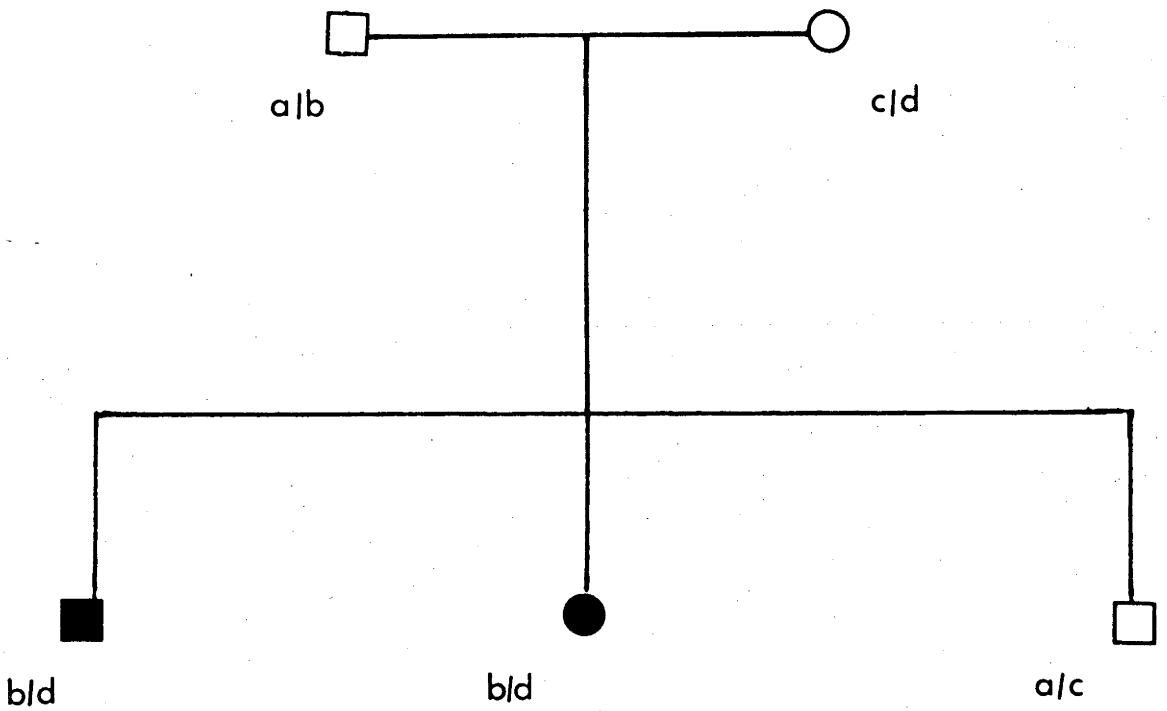
Family 1 shows $\underline{Bf}^{F1}$ to be transmitted from a normal parent to two sibs, both diabetic and $\underline{Bf}^{F1}$ positive. When HLA haplotypes with $\underline{Bf}^{F1}$ are examined, the diabetic sibs possess B18 $\underline{Bf}^{F1}$ Drw3 GLO 2. B18 being strongly linked with $\underline{Bf}^{F1}$. In both cases the heterozygote Drw 3 Drw4 is present, constituting the high risk haplotype.

Family 2 shows a diabetic parent with $\underline{Bf}^{F1}$, transmitted to two non-diabetic offspring. In the same family the diabetic sib did not possess $\underline{Bf}^{F1}$. However, when the HLA haplotypes are considered, the two non-diabetic offspring are B18 Drw3 Drw3, whereas the diabetic sib is Drw3 Drw4 $\underline{Bf}^{S}$, possessing the heterozygote which constitutes the increased combined risk.

In family 3, with normal parents, both the affected and unaffected offspring carry the $\underline{Bf}^{F1}$ factor and thus the haplotype B18 Bf $F_1$ Drw3. The above examples thus show that not all IDDM cases carry the Bf $F_1$ factor, and not in every case is the haplotype Bf $F_1$ with B18 Drw3 definitely diabetogenic.

The number of alleles, at one or more loci, required for the manifestation of IDDM continues to be debated. Based on the hypotheses that Drw3, Drw4, in combination, imply the presence of two IDDM susceptibility alleles, the segregation of Bf $F_1$ in the presence of either of these will be examined. Since Bf $F_1$ is strongly associated with IDDM (evidenced from this and other studies) and confers a risk of 6.2 in the general population, then it is expected that this allele will accompany the high risk alleles in a haplotype combination for the manifestation of IDDM in some cases.

FAMILY - 1




a = $A_{24}B_5w_4Cw_4Drw6Bf^SGLO^2C'_2$

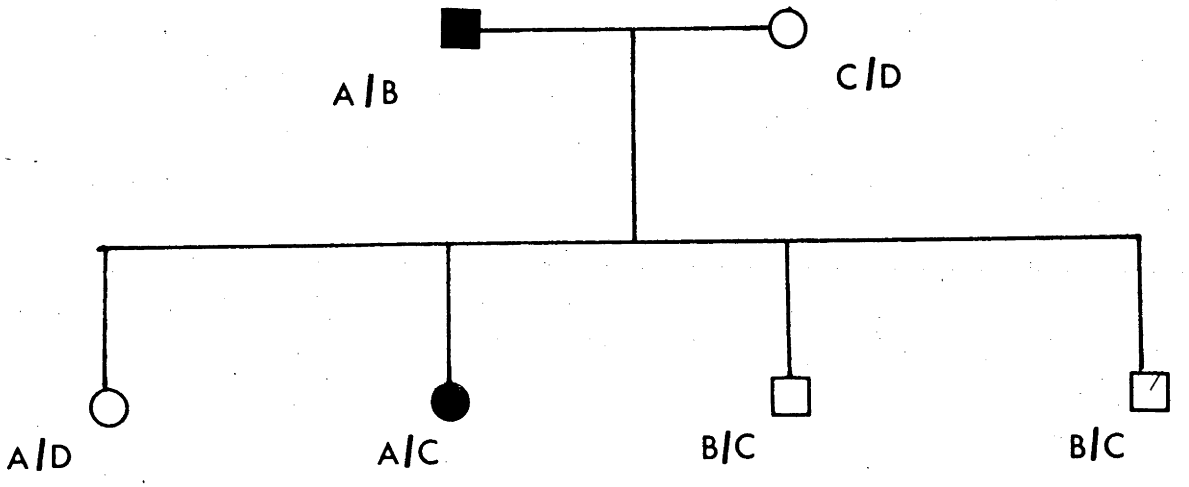
b = $A_{24}B_{12(w44)}w4\ Cw_4Drw_4Bf^SGLO^2C'_2$

c = $A_2B_{(w44)}w_4Cw_4Drw_4Bf^SGLO^2C'_2$

d = $A_2\mathbf{B}_{18}w_6\text{-}Drw_3\mathbf{B}fF_1GLO^2C'_2$

Family 1: Two children with insulin-dependent diabetes mellitus. Males designated by squares, females by circles. HLA; Bf, C´2 and GLO types are shown for each individual. Refer text for description.

FAMILY - 2




A = $A_{28}$-$w_6$-$Drw_4Bf^SGLO^1C'_2$

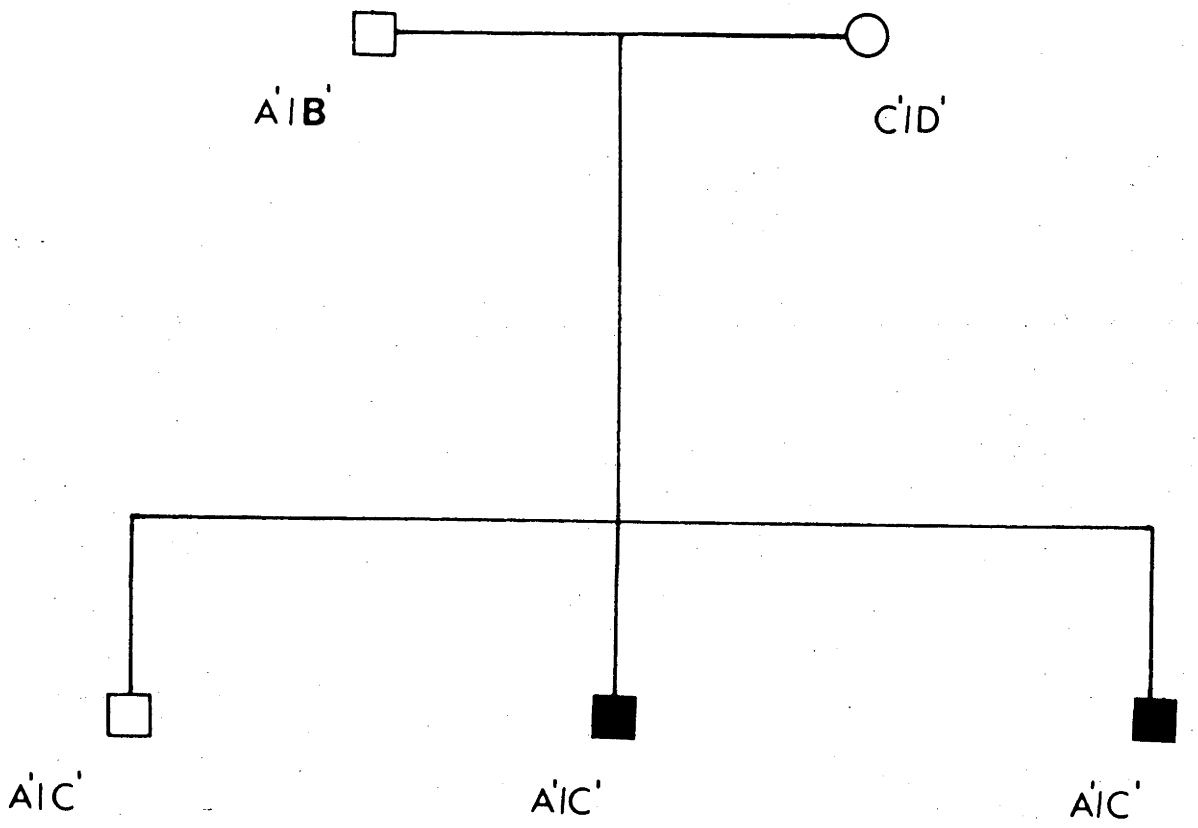
B = $A_{30}B_{18}w_6$-$Drw_3BfF_1GLO^1C'_2$

C = $A_1B_8Cw_4w_6Drw_3Bf^SGLO^2C'_2$

D = $A_2B(w_{44})Cw_4w_4Drw_6Bf^fGLO^2C'_2$

Family 2: One parent and child affected by IDDM. Males represented by squares, females by circles. HLA, Bf, C´2 and GLO types are shown for each individual. Refer text for description.

FAMILY - 3




$A' = A_{30} B_{18} D_3 Bf^{F_1} GLO^1$

$B' = A_3 \; B_7 \; D_3 Bf^S GLO^2$

$C' = A_{30} B_{40} D_4 Bf^S GLO^1$

$D' = A_3 \; B_7 \; D_1 Bf^S GLO^2$

Family 3: Two male children affected by IDDM one (male) non-affected. HLA, Bf and GLO types are given for each individual. C´2 types are not included in this family.

If the heterozygote Drw3,Drw4 reflects the presence of two IDDM susceptibility alleles, then either Drw3X (where X is any other antigen except 4) or Drw3 Drw3 will have one susceptibility allele and Drw4X (where X is any antigen except 3) or Drw4 Drw4 will have the other. From the present observations examples of families similar to Family No.2 and Family No.3 above were not uncommon. The sibs, homozygous for Drw3 and positive for Bf $F_1$ are unaffected (eg. family 2). That is they have one susceptibility allele with the additional risk factor Bf $F_1$ but do not manifest IDDM. The other child in the family with Drw3X or Drw3 and BfS is diabetic, and in family 3 the affected and unaffected have the same haplotype combination. Since the non- affected sibs also have the two high risk factors, and are in the ages between 9-14 years, there is a possibility that they may still develop diabetes at a later stage.

If non-familial cases, i.e. where only one sib is affected, are investigated separately, we can observe the segregation of the $Bf^{F1}$ allele. To define a non-familial case, the criteria used were outlined by Svejgaard *et al* (1980); i.e. a family having no first degree relatives affected and where there is at least one healthy sib at the age of 20 years or more. Only one family from the present study fulfils these criteria. Examination of the Bf $F_1$ factor in the affected child showed the following combination B18,Bf $F_1$,Drw3,i.e. heterozygous at the D locus. The emphasis thus rests on the above haplotype being of risk for a specific type of IDDM. Children with this combination (B18,Bf $F_1$,Drw3), may be different from those without this combination in the familial and non-familial cases, conferring the appropriate genetic susceptibility to the individual.

Since the RR for Bf $F_1$ is higher than for the HLA-B locus alleles, its independent effect in conferring risk is likely (Dornan *et al*, 1980) and the possibility of one of the IDDM susceptibility alleles being closely linked to $\underline{Bf}^{F1}$ (Raum *et al*, 1979) is great.

Complement component C´2

The results of typing for complement C´2 on the same IDDM population studied by Kirk *et al* (1980) have been used in the following discussion of the analysis with the Bf polymorphism.

The codominant alleles controlling structural variants of complement C´2 are present in Europeans. Three phenotypes C´2 1-1, 2-1 and 2-2 are possible, of which C´2 2-2 is rare and was not observed in the study. In the IDDM series, an increase in the C´2 2-1 phenotypes was observed, 13.5% compared to 5% and 6.9% in NIDDM and control series (table 3.1.8 taken from Kirk *et al*, 1980) comparing well with results of Alper (1976) ; Olaison *et al* (1978). The relative risk in IDDM for persons carrying a $C´2^2$ gene is 2.2. Chi-square for heterogeneity of $C´2^2$ gene frequency between IDDM and controls: $\chi^2_1 = 4.4$ $P<0.02$; $\chi^2_1$ IDDM vs. NIDDM = 8.1 $P<0.001$.

The locus controlling the synthesis of C´2 has been localised to the HLA region (Fu *et al*, 1974) and further studies showed a close localization to the HLA-B locus (Raum *et al*, 1976; Alper and Rosen, 1976). For this reason, an analysis for associations, or interaction, between alleles of C´2 and Bf, which is also close to the HLA-B locus, was carried out.

Table 3.1.8a shows the distribution of Bf phenotypes in

Table 3.1.8

Distribution of C´2 types in diabetes and control populations of Melbourne

| Series | C´2 Phenotypes | | No. tested | Per cent Gene frequency $\underline{C´2}^2$ |
|---|---|---|---|---|
| | 1-1 | 2-1 | | |
| Controls | 175(93.1%) | 13(6.9%) | 188 | 3.5% |
| IDDM | 179(86.5%) | 28(13.5%) | 207 | 6.8% |
| NIDDM | 191(95%) | 10(5%) | 201 | 2.5% |

Heterogeneity of gene frequency for IDDM vs. controls:

$$\chi^2_1 = 4.4 \quad 0.05<P<0.02$$

IDDM vs. NIDDM:

$$\chi^2_1 = 8.1 \quad 0.01<P<.001$$

Table 3.1.8a

Distribution of Bf phenotypes in relation to the C´2 phenotypes among the IDDM and controls series of Melbourne. The expected values are given in parenthesis

| Bf | IDDM | | Total | CONTROL | | Total |
|---|---|---|---|---|---|---|
| | C´2 | | | C´2 | | |
| | 1-1 | 2-1 | | 1-1 | 2-1 | |
| S | 121 (118.8) | 17 (19.1) | 138 | 99 (99.2) | 8 (7.7) | 107 |
| FS | 28 (26.7) | 3 (4.3) | 31 | 43 (43.6) | 4 (3.4) | 47 |
| F | 3 (3.4) | 1 (0.6) | 4 | 4 (3.7) | 0 (0.29) | 4 |
| SF1 | 17 (18.9) | 5 (3.0) | 22 | 3 (2.7) | 0 (0.21) | 3 |
| FF1 | 2 (1.7) | 0 (0.3) | 2 | 2 (1.8) | 0 (0.14) | 2 |
| SS1 | 3 (4.3) | 2 (0.7) | 5 | 3 (2.7) | 0 (0.21) | 3 |
| TOTAL | 174 | 28 | 202 | 154 | 12 | 166 |

relation to the C´2 phenotypes among IDDM and control individuals. From the expected values (given in parenthesis) it is clear that the distribution of Bf and C´2 are independent of each other. The BfS is present in all the C´2 2-1 individuals. Of the rare factors, among the IDDM individuals, 7 of 28 with $S_1$ or Bf $F_1$ have C´2 2-1 phenotypes, as compared to none in the control series. However, from the pairwise analysis of the Bf and C´2 2 factors none of the chi-square values obtained reach the 5% level of significance (table 3.1.8b). Therefore, although there is an apparent association between Bf rare factors and C´2 2 in patients, which probably has some biologic importance, it needs further studies of these associations.

The locus for C´2 and GLO are on the same chromosome and, in order to test any disequilibria between them, examination of the joint phenotypic distribution was carried out (table 3.1.8c). Following a $\chi^2$ test for the respective series, the factors were found to be distributed at random and no genetic disequilibrium was observed.

Non-chromosome 6 markers

In addition to the markers controlled by genes on chromosome 6 discussed in detail above, a further 10 genetic systems were investigated controlled by loci not linked to chromosome 6. Three of these systems are detectable in serum: the remaining seven are red cell enzymes.

Table 3.1.8b

Chi-square values for pairwise analysis of associations between individuals positive for Bf and $C_2'2$ factors in IDDM and control series

| FACTORS | IDDM | CONTROLS |
|---|---|---|
| Bf | $C_2'2$ | $C_2'2$ |
| S | 0.04 | 0.24 |
| F | 0.35 | 0.04 |
| S1 | 2.93 | 0.12 |
| F1 | 1.10 | 0.20 |
| S1&F1 | 3.00 | 0.37 |

Table 3.1.8c

The distribution of GLO and C´2 phenotypes among IDDM and controls of a Melbourne population

(expected values are shown in parenthesis)

| GLO | IDDM | | Total | CONTROL | | Total |
|---|---|---|---|---|---|---|
| | C´2 | | | C´2 | | |
| | 1-1 | 2-1 | | 1-1 | 2-1 | |
| 1-1 | 27 (26.6) | 4 (4.4) | 31 | 23 (23.4) | 2 (1.6) | 25 |
| 2-1 | 72 (74.7) | 15 (12.2) | 87 | 73 (71.9) | 4 (5.0) | 77 |
| 2-2 | 48 (46.0 | 5 (7.4) | 53 | 46 (46.7) | 4 (3.2) | 50 |
| TOTAL | 147 | 24 | 171 | 142 | 10 | N= 152 |

Serum proteins

The Group Specific Component (Gc)

Table 3.1.9 gives the distribution of Gc types and gene frequencies for patients and controls in the Melbourne series. The decrease of 4.8 % in the $Gc^2$ frequency among NIDDM patients tends towards significance. ($\chi^2_1 = 3.06$; $0.05>P>0.10$). In contrast the Gc gene frequency for the IDDM patients is not significantly different from the controls. In the MIDDM series the frequency of $Gc^2$ is 3.2% higher than in controls, though the difference is not significant ($\chi^2_1 = 0.62$: $P>0.5$).

An earlier study by Cleve (1966) showed a decrease in the $Gc^2$ frequency among German diabetic patients (insulin status not disclosed) less than 60 years of age, with moderate or severe arteriosclerosis of the peripheral vessels. Jörgensen and Hopfer (1967) also noted a decrease of the $Gc^2$ allele in their study. However, Berg *et al*, (1967) in the same year in a study of patients aged 14-86 in Oslo, did not find any significant difference.

It is of interest that the difference for NIDDM patients in the present study is even more marked when only the patients with a north European origin are considered (table 3.1.9a), and even though the MIDDM frequencies are still in the opposite sense, if all mature onset patients are combined (NIDDM and MIDDM) the Gc phenotype distribution is still significantly different from that of the controls ($\chi^2_2 = 6.10$: $P<0.02$). The Gc system therefore is clearly one which needs further study in relation to diabetes and this will be discussed further at a later stage of

Table 3.1.9

Distribution of Group Specific Component (Gc) phenotypes and gene frequency in the total diabetic and control population of Melbourne

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $\underline{Gc}^1$ | $\underline{Gc}^2$ |
|---|---|---|---|---|---|---|
| Controls | 168 | 148 | 42 | 358 | 0.6760 | 0.3240 |
| IDDM | 110 | 103 | 23 | 236 | 0.6843 | 0.3157 |
| NIDDM | 111 | 104 | 10 | 225 | 0.7244 | 0.2755 |
| MIDDM | 33 | 37 | 10 | 80 | 0.6438 | 0.3562 |

Heterogeneity of phenotypes in controls vs. IDDM:

$\chi^2_2 = 0.71$ $P>.75$

vs. NIDDM:

$\chi^2_2 = 6.26$ $0.05<P<0.02$

gene frequency in controls vs. NIDDM:

$\chi^2_1 = 3.06$ $0.05>P>0.10$

Presence of $\underline{Gc}^2$ vs. absence Woolf's chi-square = 0.36 n.s.

Table 3.1.9a

Distribution of Group Specific Component and gene frequency in diabetic and controls in a North European population of Melbourne

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $\underline{Gc}^1$ | $\underline{Gc}^2$ |
|---|---|---|---|---|---|---|
| Controls | 75 | 61 | 23 | 159 | 0.6635 | 0.3365 |
| IDDM | 100 | 88 | 17 | 205 | 0.7024 | 0.2980 |
| NIDDM | 87 | 74 | 7 | 168 | 0.7411 | 0.2619 |
| MIDDM | 26 | 27 | 9 | 62 | 0.6370 | 0.3630 |

Test for heterogeneity of phenotypes in IDDM vs. controls:

$\chi^2_2 = 0.71$ n.s.

in MIDDM+NIDDM vs. controls:

$\chi^2_2 = 6.10$ $P<0.02$

in NIDDM vs. controls:

$\chi^2_2 = 10.5$ $P<0.001$

gene frequencies in NIDDM vs. controls:

$\chi^2_1 = 4.3$ $0.05<P<0.02$

in MIDDM+NIDDM vs. controls:

$\chi^2_1 = 0.71$ n.s.

Woolf's RR=0.40; $\chi^2_1 = 25.1$ $P>.001$

for NIDDM vs. control

this thesis.

Haptoglobin (Hp)

Table 3.1.10 shows the distribution of phenotypes and gene frequencies of haptoglobin in patients and control series. A decrease of 3.6% and 3.1% in the $Hp^2$ allele frequency is noted in the IDDM and MIDDM series. But in NIDDM there is an increase of 3.2% in $Hp^2$ allele frequency, compared to controls. These differences are not statistically significant.

Early studies have shown a non-significant increase of Hp 1-1 phenotypes among diabetics in some series (Berg *et al*, 1967) and a decrease of Hp 1-1 in others (Simpson *et al*, 1962; Jörgensen and Hopfer 1967). In this study, there is a decrease of 4.7% in the Hp 2-2 phenotypes in the IDDM series when compared to controls, but there is no significant heterogeneity in phenotype distribution between the two series ($\chi^2_2 = 1.47$ $P<0.5$).

It has been hypothesized, on the basis of studies of Hp distribution in other diseases and normal controls that disturbances of this kind are due to the effects of selection on the alleles at the Hp locus (Workman *et al*, 1963; Peacock, 1966). However, it seems clear that no definite conclusion can be drawn until more data on larger samples, show consistent increase or decrease in Hp alleles in diabetic individuals.

Transferrin (Tf)

Variants of the iron-binding protein, transferrin are uncommon in Europeans with approximately 98% of persons having

Table 3.1.10

Distribution of haptoglobin (Hp) phenotypes and gene frequency in the total diabetic and control population of Melbourne

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $Hp^1$ | $Hp^2$ |
|---|---|---|---|---|---|---|
| Control | 52 | 172 | 149 | 373 | 0.3700 | 0.6300 |
| IDDM | 40 | 116 | 85 | 241 | 0.4066 | 0.5934 |
| NIDDM | 29 | 95 | 102 | 226 | 0.3385 | 0.6620 |
| MIDDM | 15 | 35 | 31 | 81 | 0.4012 | 0.5990 |

Heterogeneity of phenotypes in controls vs. IDDM:

$$\chi^2_2 = 1.47 \quad P<0.50$$

controls vs. NIDDM

$$\chi^2_2 = 1.41 \quad P<0.50$$

gene frequency NIDDM vs. controls

$$\chi^2_1 = 1.22 \quad P>0.30$$

IDDM vs. NIDDM+MIDDM

$$\chi^2_2 = 3.43 \quad 0.10>P>0.25$$

only the common transferrin TfC. The present study shows this to be true for both the diabetic and control series (Table 3.1.11) and there are no significant differences between patient and controls in the number or gene frequency for rare alleles.

Ceruloplasmin (Cp)

The distribution of the protein ceruloplasmin, an $\alpha_2$ globulin is known to have fast moving electrophoretic variants which are of very rare occurrence in most populations with the exception of American Negroes. Cp BB is the most common phenotype and exists with a frequency of 99% in non-negro populations (Shreffler *et al*, 1967). In the present investigation,no variants were observed and there was 1.00% distribution of the $Cp^B$ allele in all the populations examined. Therefore, this system will be excluded from discussions in this and the subsequent chapters.

Non -chromosome 6 markers - Red cell enzymes

Esterase-D (EsT-D)

This is a two allele codominant system,for which three phenotypes, Est-D 1-1,2-1 and2-2 exist. Est-D 2-2 is relatively rare among Europeans. Table 3.1.12 gives the distribution of Est-D phenotypes and gene frequencies for the present study.

In the diabetic population, there is a non-significant decrease in Est-$D^2$ frequency of 1.9%, 2.2% and 1.2% in the IDDM, NIDDM and MIDDM series compared to control series. The IDDM series shows a significant absence of the Est-D 2-2 phenotype when compared to controls and mature onset diabetics. This may be due to a sampling error and further study is needed.

Table 3.1.11

Distribution of transferrin (Tf) phenotypes and gene frequency in the diabetic and control population of Melbourne

| Series | Phenotypes CC | $CD_1$ | $CB_2$ | No. Tested | Gene Frequency $\underline{Tf}^C$ | $\underline{Tf}^{D1}$ | $\underline{Tf}^{B2}$ |
|---|---|---|---|---|---|---|---|
| Controls | 361 | 1 | 6 | 368 | 0.9918 | 0.0014 | 0.0082 |
| IDDM | 248 | 0 | 2 | 250 | 0.9960 | - | 0.0040 |
| NIDDM | 227 | 1 | 1 | 229 | 0.9978 | 0.0022 | 0.0022 |
| MIDDM | 80 | 0 | 1 | 81 | 0.9938 | 0.0062 | 0.0062 |

Heterogeneity of phenotypes in IDDM vs. controls:

$\chi^2_2 = 1.19$ $P > 0.50$

NIDDM vs. controls:

$\chi^2_2 = 2.36$ $P < 0.25$

IDDM vs. NIDDM+MIDDM:

$\chi^2_2 = 0.66$ n.s.

Table 3.1.12

Distribution of Esterase-D phenotypes and gene frequencies in the total diabetic and control population of Melbourne

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency Est-D$^1$ | Est-D$^2$ |
|---|---|---|---|---|---|---|
| Control | 290 | 79 | 10 | 379 | 0.8694 | 0.1306 |
| IDDM | 193 | 55 | 0 | 248 | 0.8891 | 0.1109 |
| NIDDM | 187 | 37 | 4 | 228 | 0.9013 | 0.1086 |
| MIDDM | 62 | 17 | 1 | 80 | 0.8813 | 0.1187 |

Test for heterogeneity of phenotypes in controls vs. IDDM:

$$\chi^2_2 = 5.67 \quad 0.05>P>0.10$$

controls vs. NIDDM+MIDDM:

$$\chi^2_2 = 2.29 \quad P<0.25$$

gene frequency in controls vs. NIDDM+MIDDM:

$$\chi^2_1 = 2.30 \quad P<0.10$$

phenotypes in IDDM vs. NIDDM:

$$\chi^2_2 = 6.35 \quad 0.05<P<0.025$$

gene frequency in IDDM vs. NIDDM:

$$\chi^2_1 = 0.36 \quad \text{n.s.}$$

Williams and Cartwright (1978) studied Est- D in a diabetic population based on age categorization, but no significant difference in any age group was noted. To test whether age categorization would show a difference in the distribution of Est-D phenotypes, the present series was also subjected to a similar analysis. The results, like the previous study, were negative, supporting the observation of the above authors.

Acid phosphatase (PHs)

The phenotypic distribution in the diabetic and control population is shown in table 3.1.13a. There is a decrease of 3% in the frequency of the $PHs^C$ allele among the IDDM patients when compared to controls and of 1.7 and 0.6% in the two mature onset diabetic series. When tested for heterogeneity of gene frequency and phenotypes, there was no significant difference in any of the series.

One previous study (Lucarelli *et al*, 1978) reported a difference in the acid phosphatases among diabetics in Rome when the population was divided on the basis of sex. Among males, the CB phenotypes were under-represented when compared with controls. In the present series, when divided by sex, there is also a deficiency of CB phenotypes among males in the IDDM series (0% vs. 8.9% in controls). The situation is reversed in females and combining both sexes the CB phenotype is 5.1% in IDDM and 6.8% in controls, the difference being non-significant (table 3.1.13b).

Phosphoglucomutase I and II

At the $PGM_2$ locus, no variation was observed in the control

Table 3.1.13a

Distribution of acid phosphatase phenotypes and gene frequency in total diabetic and control population of Melbourne

| Series | Phenotypes | | | | | No. Tested | Gene Frequency | | |
|---|---|---|---|---|---|---|---|---|---|
| | AA | AB | BB | CA | CB | | $\underline{PHs}^A$ | $\underline{PHs}^B$ | $\underline{PHs}^C$ |
| Control | 46 | 154 | 135 | 22 | 25 | 382 | 0.3508 | 0.5877 | 0.0615 |
| IDDM | 30 | 112 | 91 | 5 | 11 | 249 | 0.3604 | 0.6124 | 0.0321 |
| NIDDM | 21 | 97 | 89 | 4 | 16 | 227 | 0.3150 | 0.6410 | 0.0441 |
| MIDDM | 8 | 30 | 34 | 4 | 5 | 81 | 0.3086 | 0.6358 | 0.0556 |

Test for heterogeneity of phenotypes in IDDM vs. controls:

$\chi^2_4 = 6.62 \quad 0.20<P<0.10$

NIDDM+MIDDM vs. controls:

$\chi^2_4 = 6.00 \quad 0.20<P<0.10$

gene frequency in NIDDM+MIDDM vs. controls:

$\chi^2_1 = 1.55 \quad$ n.s.

IDDM vs. NIDDM+MIDDM:

$\chi^2_1 = 3.64 \quad P < 0.05$

Table 3.1.13 b

Distribution of acid phosphatase phenotypes and gene frequency in male and female IDDM and controls of Melbourne

| | Phenotypes | | | | | | Gene frequency | | |
|---|---|---|---|---|---|---|---|---|---|
| Males[§] | AA | AB | BB | CA | CB | TOTAL | $PHs^A$ | $PHs^B$ | $PHs^C$ |
| Controls | 12 | 56 | 42 | 2 | 11 | 123 | 0.3333 | 0.6138 | 0.1028 |
| IDDM | 4 | 21 | 11 | 2 | 0 | 38 | 0.4079 | 0.5660 | 0.0263 |
| Females[+] | | | | | | | | | |
| Controls | 7 | 29 | 24 | 6 | 2 | 68 | 0.3603 | 0.5808 | 0.1008 |
| IDDM | 5 | 19 | 13 | 0 | 4 | 41 | 0.3537 | 0.5980 | 0.0490 |

[§] Test for heterogeneity of gene frequency A and B vs. C: $\chi^2_1 = 0.87$ n.s.

[+] gene frequency A and B vs. C: $\chi^2_1 = 3.3$ $0.1 < P < 0.05$

and diabetic series.

At locus 1, shown in table 3.1.14a, the frequency of $PGM_1$ in controls is comparable and agrees well with the published frequencies for Europeans. In the NIDDM and MIDDM, there is an increase of 3.8% and 7.3% in the phenotype PGM 1-1 frequency as compared to controls. When tested for heterogeneity of phenotypes and gene frequency in the total series, however, this was not significant.

When only the North European population is considered (table 3.1.14b) there is an increase of 11.9% and 9.9% in the PGM 1-1 among the NIDDM and MIDDM series respectively. There is a corresponding decrease in the PGM 2-1's in both and PGM 2-2 in the NIDDM series and this is reflected in a decrease in the $\underline{PGM}_1^2$ gene frequency among the mature onset patients. The difference in the gene frequencies in NIDDM vs. controls shows significance at the 2 per cent level.

One earlier study noted an increase of $\underline{PGM}_1^1$ among the older age group of unaffected white South African women (mean age 82.7 years) (Gordon and Riser, 1966). It is possible, therefore, that the increase of 1-1 phenotypes found in the present study, which is significant among patients of North European origin, may be due to an age effect. Of NIDDM and MIDDM Melbourne patients, for which relevant information was available, 25.9% were in the age group 70 years and over (the distribution of per cent gene frequency based on age groups is given in table 3.1.14c. Those of 70 years and above show a 5% decrease of $\underline{PGM}_1^2$ allele as compared to the 40-69 years age group), whereas none of the controls were aged above 60 years. The present study suggests that further

Table 3.1.14a.

Distribution of phosphoglucomutase (PGM) (locus 1) phenotypes and gene frequency in total diabetic and control population of Melbourne

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $\underline{PGM}_1^1$ | $\underline{PGM}_1^2$ |
|---|---|---|---|---|---|---|
| Control | 221 | 128 | 31 | 380 | 0.7500 | 0.2500 |
| IDDM | 141 | 91 | 18 | 250 | 0.7460 | 0.2540 |
| NIDDM | 140 | 73 | 13 | 226 | 0.7810 | 0.2190 |
| MIDDM | 53 | 21 | 7 | 81 | 0.7840 | 0.2160 |

Heterogeneity of phenotypes in IDDM vs. control:

$\chi^2_2 = 0.45$ n.s.

MIDDM+NIDDM vs. controls:

$\chi^2_2 = 1.76$ $P>0.50$

IDDM vs. NIDDM+MIDDM:

$\chi^2_2 = 2.48$ $P<0.25$

gene frequency in NIDDM+MIDDM vs. control:

$\chi^2_1 = 1.94$ $P<0.25$

Table 3.1.14b.

Distribution of phosphoglucomutase I phenotype and gene frequency in the diabetic and control North European population of Melbourne

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $\underline{PGM}_1^1$ | $\underline{PGM}_1^2$ |
|---|---|---|---|---|---|---|
| Control | 95 | 63 | 13 | 171 | 0.7400 | 0.2602 |
| IDDM | 117 | 82 | 12 | 211 | 0.7488 | 0.2512 |
| NIDDM | 112 | 50 | 6 | 168 | 0.8155 | 0.1850 |
| MIDDM | 36 | 15 | 4 | 55 | 0.7910 | 0.2090 |

Heterogeneity of phenotypes in IDDM vs. controls:

$\chi^2_2 = 0.34$ $P<0.8$

NIDDM vs. control:

$\chi^2_2 = 5.40$ $0.10<P<0.05$

combined MIDDM-NIDDM vs. controls:

$\chi^2_2 = 4.40$ $P<0.10$

gene frequency in NIDDM vs. controls:

$\chi^2_1 = 5.58$ $P>0.025$

Woolf's RR 1.55 $\chi^2_1 = 5.56$ $0.01>P>0.025$

Table 3.1.14c.

Distribution of $PGM_1$ per cent gene frequency in different age groups among the NIDDM series of Melbourne

| | $PGM_1^1$ | $PGM_1^2$ |
|---|---|---|
| 40-69 yrs | 78.04 | 21.96 |
| 70 and over | 83.20 | 16.80 |
| All ages | 80.60 | 19.43 |

investigation of this phenomenon is needed.

Glutamic pyruvic transaminase (GPT)

The data for this study in diabetic and control are given in table 3.1.15. In all three diabetic series, there is a decrease of GPT 1-1 phenotypes when compared to controls and a corresponding increase in the heterozygotes as well as homozygotes in cases of NIDDM and MIDDM, but these differences are not significant when tested for heterogeneity of phenotypes. However, when NIDDM and MIDDM are combined there is a significant decrease in the $GPT^1$ gene frequency and corresponding increase in $GPT^2$ compared to controls ($\chi^2_1 = 3.90$ $P = 0.05$). Again further study of this phenomenon is required.

Other enzyme systems

Three other polymorphic red cell enzyme systems were investigated during the present study: 6-phosphogluconate dehydrogenase (6PGD) adenosine deaminase (ADA) and adenylate kinase (AK). The phenotype distributions and gene frequencies are given respectively in tables 3.1.16, 3.1.17 and 3.1.18. None of these systems revealed significant differences between controls and any of the patient series for either phenotype or gene frequency distributions.

Distance analysis

The comparison of the different subgroups within a population, based on genetic markers, gives information at each locus under study, as has been described. In order to gauge the vari-

Table 3.1.15

Distribution of glutamic pyruvic transaminase (GPT) phenotypes and gene frequency in the total diabetic and control population of Melbourne

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $\underline{GPT}^1$ | $\underline{GPT}^2$ |
|---|---|---|---|---|---|---|
| Control | 63 | 89 | 37 | 189 | 0.5688 | 0.4312 |
| IDDM | 64 | 128 | 44 | 236 | 0.5424 | 0.4576 |
| NIDDM | 55 | 117 | 49 | 221 | 0.5136 | 0.4864 |
| MIDDM | 17 | 41 | 20 | 78 | 0.4808 | 0.5200 |

Heterogeneity of phenotypes in control vs. IDDM:

$$\chi^2_2 = 2.80 \quad P<0.25$$

control vs. NIDDM+MIDDM:

$$\chi^2_2 = 5.12 \quad 0.05>P>0.1$$

gene frequency in controls vs. NIDDM+MIDDM:

$$\chi^2_1 = 3.90 \quad P = 0.05$$

control vs. NIDDM:

$$\chi^2_1 = 2.10 \quad P<0.10$$

phenotypes in IDDM vs. NI+MIDDM:

$$\chi^2_2 = 1.60 \quad P<0.25$$

Table 3.1.16

Distribution of 6-Phosphogluconate dehydrogenase (6PGD) phenotypes and gene frequencies in the total diabetic and control population of Melbourne

| Series | Phenotypes AA | AC | CC | No. Tested | Gene Frequency 6PGD[a] | 6PGD[c] |
|---|---|---|---|---|---|---|
| Control | 366 | 16 | 0 | 382 | 0.9791 | 0.0210 |
| IDDM | 233 | 16 | 1 | 249 | 0.9640 | 0.0361 |
| NIDDM | 219 | 10 | 0 | 229 | 0.9782 | 0.0218 |
| MIDDM | 78 | 2 | 0 | 80 | 0.9875 | 0.0125 |

Heterogeneity of phenotypes in IDDM vs. controls:

$$\chi^2_2 = 3.03 \quad P<0.20$$

gene frequency in IDDM vs. controls

$$\chi^2_1 = 3.20 \quad 0.05>P>0.1$$

phenotypes in IDDM vs. NIDDM+MIDDM:

$$\chi^2_2 = 3.68 \quad P>0.20$$

Table 3.1.17

Distribution of adenosine deaminase (ADA) phenotypes and gene frequency in the total diabetic and control population of Melbourne

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $\underline{ADA}^1$ | $\underline{ADA}^2$ |
|---|---|---|---|---|---|---|
| Controls | 347 | 34 | 0 | 381 | 0.9554 | 0.0446 |
| IDDM | 190 | 25 | 0 | 215 | 0.9419 | 0.0581 |
| NIDDM | 184 | 17 | 0 | 201 | 0.9580 | 0.0420 |
| MIDDM | 64 | 4 | 0 | 68 | 0.9706 | 0.0300 |

Heterogeneity of phenotypes in IDDM vs. controls:

$\chi^2_2 = 1.31$ $P<0.50$

NIDDM+MIDDM vs. controls:

$\chi^2_2 = 0.31$ $P>0.90$

NIDDM+MIDDM vs. IDDM

$\chi^2_2 = 2.44$ $P = 0.30$

gene frequency in NIDDM+MIDDM vs. IDDM

$\chi^2_1 = 0.89$ n.s.

Table 3.1.18

Distribution of adenylate kinase (AK) phenotypes and gene frequency in the total diabetic and control population of Melbourne

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $\underline{AK}^1$ | $\underline{AK}^2$ |
|---|---|---|---|---|---|---|
| Controls | 353 | 26 | 1 | 380 | 0.9632 | 0.0368 |
| IDDM | 241 | 8 | 0 | 249 | 0.9839 | 0.0321 |
| NIDDM | 208 | 20 | 0 | 228 | 0.9561 | 0.0439 |
| MIDDM | 74 | 6 | 0 | 80 | 0.9625 | 0.0375 |

Heterogeneity of phenotypes in IDDM vs. controls:

$$\chi^2_2 = 3.60 \quad P>0.20$$

NIDDM+MIDDM vs. controls:

$$\chi^2_2 = 1.54 \quad P>0.50$$

gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 0.85 \quad n.s.$$

ation, or similarity at a number of loci, a composite measure which could represent the relationships of the groups within a population is desirable. In order to achieve this, on the basis of gene frequency data, the population in the present study were subjected to a multivariate analysis using the Biokin genetic distance method of Morton (1970). The method and program used for this purpose has been described in Chapter 2.

The results obtained on the basis of analysing the data for 13 genetic marker systems are depicted in figures 3.1.1a and 3.1.1b by an eigenvector representation and a dendrogram.

From the previous discussions of the results, among the important markers which revealed significance among specific disease groups were Bf alleles. The distribution of some of the Bf genes in the IDDM group was significantly higher than the two mature diabetic and control series. Subsequently the strong association of the Bf factors in the IDDM series and not in NIDDM and MIDDM specifies a distinction in the types of diabetes. One other locus studied on chromosome 6, C'2, also shows an increased frequency of the $C'2^2$ allele in the IDDM series. The possibility that the differences in the gene frequencies of the markers on chromosome 6 would cause the IDDM series to be sufficiently separated from the rest of the series, is likely, and this is apparent in the dendrogram and also on the eigenvector diagram. In the case of NIDDM and MIDDM, there is a separation of the mature diabetic series treated by insulin, from those on oral tablets or diet treatment and this may be due, in part, to the disturbances in the alleles of the Gc, GPT and PGM loci, which do not occur in the IDDM

Figure 3·1·1a

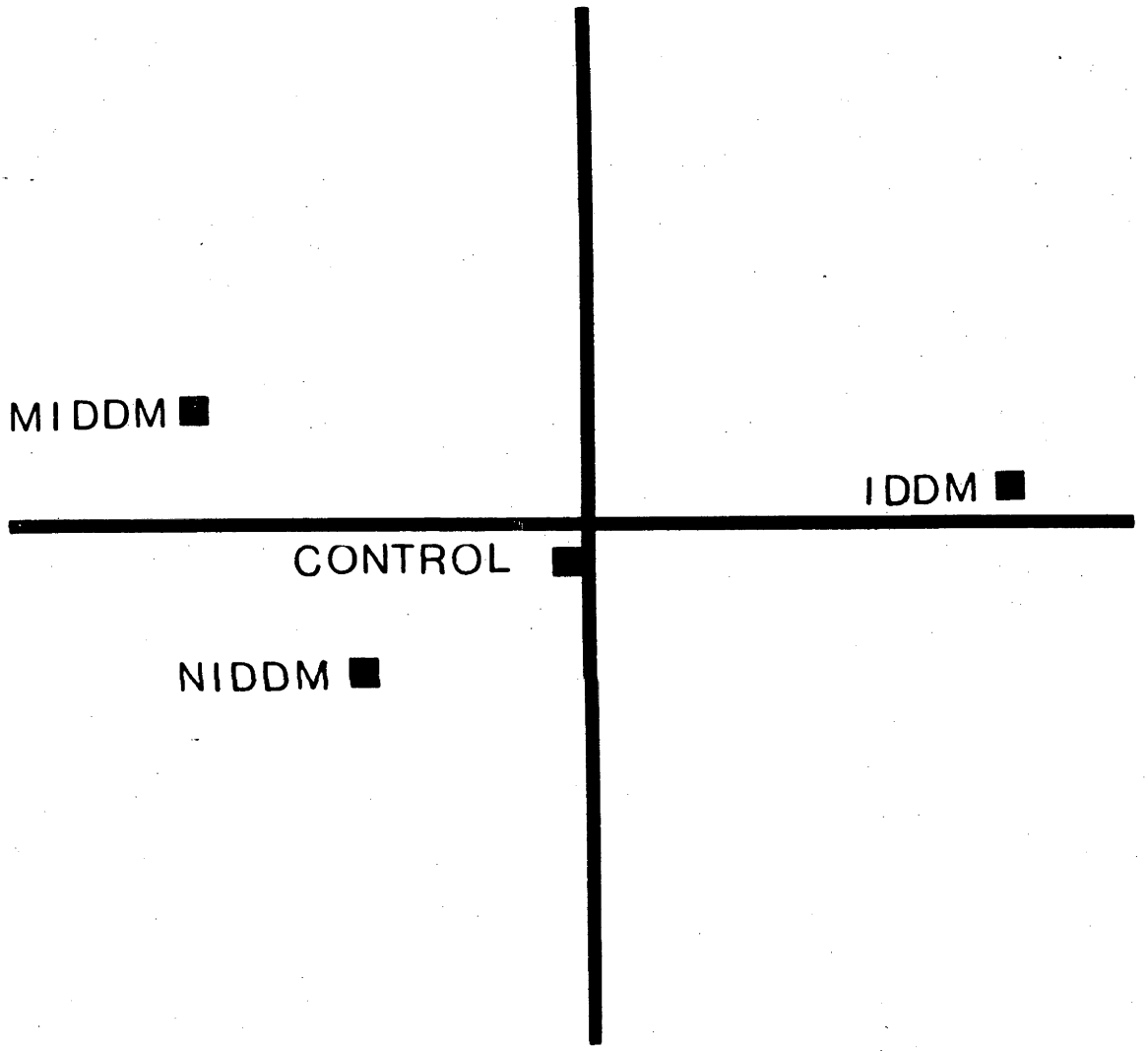



Fig. 3.1.1a. Eigenvector representation of the differences between the patients and control groups, in the Melbourne population.

Figure 3·1·1b

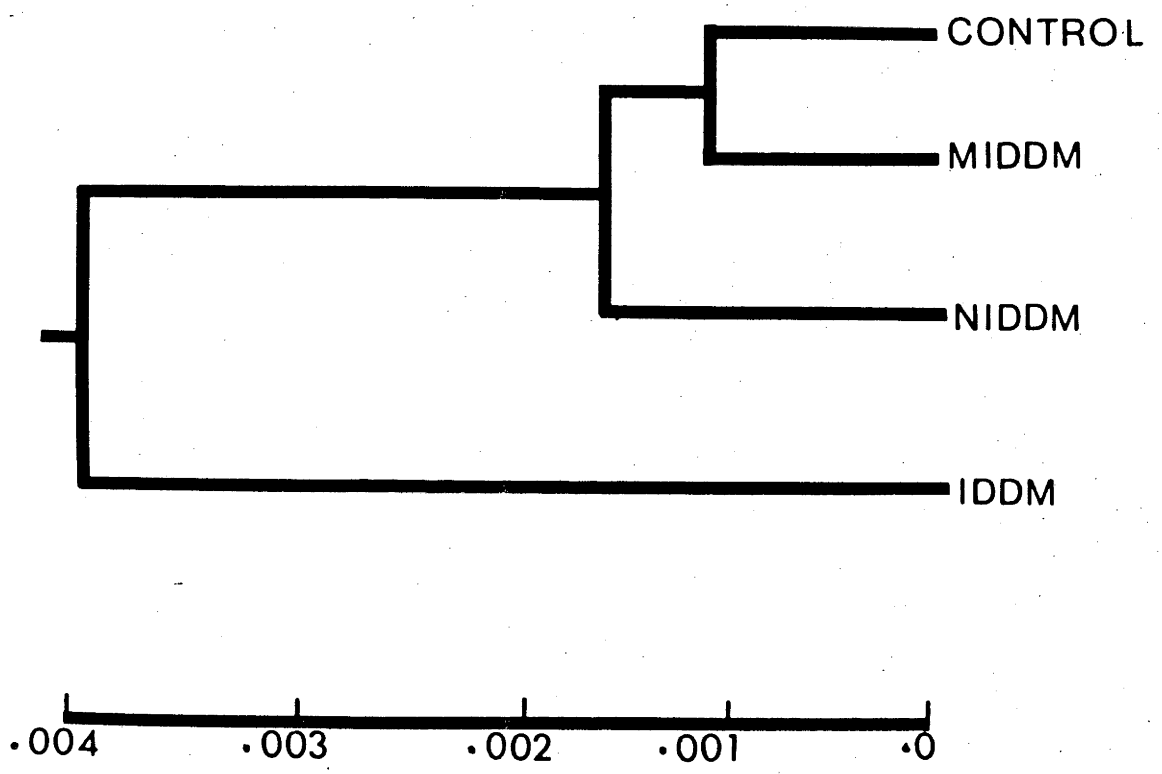



Fig. 3.1.1b. Differences between diabetic and control groups, represented by a dendrogram, in a Melbourne population.

series. However, the error due to sample size cannot be estimated.

Although the differences in the separation due to chromosome 6 markers and non-chromosome 6 markers are not depicted, the importance of both must be realized in the separation of the subgroups of diabetics from each other and the control series.

## SUMMARY

In the study of the Melbourne series of diabetics and controls, the screening of genetic markers on chromosome 6 and not on chromosome 6 have provided interesting results. In the IDDM series, a strong association of two non-HLA markers is demonstrated, that due to allele of the Bf locus and of the complement component C´2. The distinction of IDDM from the rest of the series due to these markers, show that other markers within the HLA complex may also be involved in disease processes and confer a genetic susceptibility to individuals. It is of interest also that the other chromosome 6 locus studied, glyoxalase, which is further away from the HLA region, does not contribute to the distinction between the IDDM and NIDDM or MIDDM series.

There is nothing to suggest that the Bf or C´2 loci have any direct connection with susceptibility to IDDM, it is most likely that they are important only because alleles at these loci are in linkage disequilibrium with specific disease susceptibility genes and that tests for these systems are important therefore in extending the detail of haplotypes which are characteristic

of the chromosomes carrying particular susceptibility alleles.

No definite marker in the case of NIDDM or MIDDM series can be demonstrated in this study. The significant disturbances at the Gc, GPT and PGM loci, however, indicate the importance of further study. In the case of Gc, it is known to be associated with other diseases and immune related functions for it has been suggested. Thus there is a possibility of a non-HLA, non-chromosome 6 marker being involved in the mature onset disease process. Further sudies will be necessary to draw any definite conclusions. The study also demonstrates the effects of ethnic stratification which may produce artifacts of association and indicates the importance of specifying carefully the ethnic origins of both patients and controls. In the case of the $PGM_1$ association with the NIDDM and MIDDM series, it shows also that the age of patients may be of importance as a confounding factor.

Chapter 3

# AUSTRALIAN POPULATION

## Section 2: Western Australia

The series studied was derived from three populations in W.A.:- Geraldton (n = 100), Perth (n = 33) and Kalgoorlie (n = 141). Details are given in Chapter 2. Patients were subdivided for the Melbourne series. Since the sub-division resulted in small numbers in each category, the three populations were combined for analysis.

### Chromosome 6 markers

#### Glyoxalase I (GlO)

The glyoxalase data for the combined Western Australia series are shown in table 3.2.1. In the small samples of 42 IDDM patients, the distribution of glyoxalase phenotypes shows an increase of 14.5% in GLO 1-1 as compared to controls; correspondingly there is a decrease of 13% and 1.1% in the GLO 2-1 and 2-2 phenotypes. The difference, when tested for heterogeneity, is not significant.

In the MIDDM series there is an 8.8% increase of GLO 1-1 which also is non-significant. The NIDDM series compares closely to controls with a difference of 1.6% in GLO 1-1 and a decrease of GLO 2-1 by 5%. Even when the two mature onset diabetic series (MIDDM and NIDDM) are combined the differences from controls in the two series are not significant. This is unlike the results shown in the Melbourne NIDDM series, where there is a distortion in the GLO phenotypes. It was suggested that this is due probably to the ethnic stratification of the Melbourne population. Unfortun-

Table 3.2.1

Distribution of glyoxalase (GLO) phenotypes and gene frequency in diabetic and control population of Western Australia

| Series | Phenotypes | | | No. Tested | Gene Frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{GLO}^1$ | $\underline{GLO}^2$ |
| Control | 72 | 195 | 115 | 382 | 0.4437 | 0.5563 |
| IDDM | 14 | 16 | 12 | 42 | 0.5238 | 0.4762 |
| NIDDM | 24 | 58 | 43 | 125 | 0.4240 | 0.5760 |
| MIDDM | 18 | 32 | 15 | 65 | 0.5231 | 0.4769 |

Heterogeneity of phenotypes in IDDM vs. controls:

$\chi^2_2 = 5.3$ $0.05>P>0.10$

NIDDM vs. controls:

$\chi^2_2 = 1.0$ $P<0.5$

MIDDM+NIDDM vs. controls:

$\chi^2_2 = 0.96$ n.s.

gene frequency in IDDM vs. controls:

$\chi^2_1 = 2.0$ $P<0.1$

in NIDDM + MIDDM vs. controls:

$\chi^2_1 = 0.26$ n.s.

ately information about ethnic origin of patients in the W.A. series was not available so that no analysis of this point could be made

Complement factor : Properdin factor B (Bf)

The strong association of IDDM with Bf rare factors demonstrated in the Melbourne population can also be observed in the Western Australian series. Of the 39 IDDM individuals, there are 5 (13%) with factors $BfS_1$ and $BfF_1$ (table 3.2.2) as compared to 12 (3.2%) in controls. The increase of combined $\underline{Bf}^{F1}$ and $\underline{Bf}^{S1}$ genes in the IDDM patients is significant ($\chi^2_1 = 8.25$: $0.01 < P < 0.001$).

When the age distribution is considered, the individuals with the Bf rare factors were 4, 9, 27(2) and 28 years at the time of onset. In the remaining IDDM patients the ages ranged from 14-39 years, with only 3 individuals below the age of 10 years. This higher age of diagnosis of IDDM patients in the W.A. population, as compared with the Melbourne series, is due to the different method of ascertainment in the two localities, particularly the reliance in Melbourne on obtaining IDDM patients from a children's hospital.

There is no significant increase of the Bf rare alleles among the NIDDM and MIDDM series: $\chi^2_1 = 0.037$ (n.s.). Like the Melbourne series, there is an increase of the $\underline{Bf}^{S}$ allele in all three W.A. diabetic series, 5.2% in the IDDM and MIDDM and 4.8% in the NIDDM series. A corresponding decrease of $\underline{Bf}^{F}$ allele is also noted and is significant in the IDDM series (9.8% compared to controls) with a probability at less than the 2 per cent level. In the NIDDM and MIDDM series this decrease of the $\underline{Bf}^{F}$ allele is 5.2 and 5.1%

Table 3.2.2

Distribution of properdin factor B (Bf) phenotypes and gene frequencies in diabetic and control population of Western Australia

| Series | Phenotypes | | | | | | | No. Tested | Gene frequency | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SS | FS | FF | $S_1S$ | $F_1F$ | $F_1S$ | $FS_1$ | | $Bf^S$ | $Bf^F$ | $Bf^F1$ | $Bf^S1$ |
| Control | 248 | 100 | 20 | 3 | 3 | 5 | 1 | 380 | 0.7947 | 0.1895 | 0.0105 | 0.0053 |
| IDDM | 27 | 7 | 0 | 1 | 0 | 4 | 0 | 39 | 0.8462 | 0.0907 | 0.0512 | 0.0128 |
| NIDDM | 91 | 24 | 4 | 2 | 2 | 1 | 0 | 124 | 0.8427 | 0.1371 | 0.0120 | 0.0081 |
| MIDDM | 45 | 18 | 0 | 2 | 0 | 0 | 0 | 65 | 0.8462 | 0.1385 | 0.0000 | 0.0153 |

Heterogeneity of gene frequency in IDDM vs. controls: $Bf^F$ $\chi^2_1 = 4.7$ $0.05<P<0.02$

$Bf^S1+Bf^F1$ $\chi^2_1 = 8.3$ $0.01<P<0.001$

NIDDM vs. controls: $Bf^F$ $\chi^2_1 = 3.7$ $0.1<P<0.05$

NIDDM+MIDDM vs. controls: $Bf^F$ $\chi^2_1 = 5.0$ $0.05<P<0.02$

$Bf^F1+Bf^S1$ $\chi^2_1 = 0.03$ n.s.

respectively. When the two series are combined the difference is significant at the P = 0.05 - 0.02 level.

The association of the Bf rare factors in both the W.A. and Melbourne IDDM series confirms the importance of these as markers for susceptibility to the insulin-dependent form of the disease, at least in populations of European origin.

It is of further interest, however, that in the mature-onset patients in W.A. there is a significant decrease in the $\underline{Bf^F}$ allele. A similar decrease was present in the Melbourne series though it was not significant.

Complement Component C'2

The increased frequency of C´2 2-1 phenotype described for the Melbourne IDDM series, is also seen among the West Australian IDDM series, but does not reach a significant level (table 3.2.3). The NIDDM and MIDDM series compare well with the control series. The allele frequency for $C´2^2$ is 3.4% higher in the IDDM series and less by 1.0% in NIDDM and MIDDM respectively when compared to controls.

Analysis for association between C´2 and Bf factors was undertaken to see whether there was any disequilibrium between the factors of the two systems. Table 3.2.4 gives the observed and expected values (in parenthesis). The $\chi^2$ values obtained do not show significance and therefore the distribution of phenotypes in the two systems is independent. This compares well with the results in the Melbourne IDDM series. None of the Bf rare factors are present in the C´2 2-1 positive individuals either in the IDDM or the control series in this population.

Table 3.2.3

Distribution of complement factor C´2 phenotype and gene frequency in a diabetic and control population of Western Australia

| Series | Phenotypes C´2 | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|
| | 1-1 | 2-1 | | $C´2^1$ | $C´2^2$ |
| Controls | 175 | 13 | 188 | 0.9650 | 0.0350 |
| IDDM | 31 | 5 | 36 | 0.9310 | 0.0690 |
| NIDDM | 74 | 4 | 78 | 0.9744 | 0.0256 |
| MIDDM | 44 | 3 | 47 | 0.9680 | 0.0320 |

Heterogeneity of phenotypes in IDDM vs. controls:

$\chi^2_1 = 1.93$ $P<0.2$

gene frequency $\chi^2_1 = 1.96$ $P<0.2$

IDDM vs. NIDDM vs. controls:

$\chi^2_1 = 2.5$ $P<0.1$

IDDM vs. NIDDM+MIDDM vs. controls:

$\chi^2_1 = 2.7$ $P=0.1$

Table 3.2.4

Distribution of joint phenotypes of C´2 and Bf factors in control and IDDM population of Western Australia

| Bf | IDDM | | Total | CONTROL | | Total |
|---|---|---|---|---|---|---|
| | C´2 | | | C´2 | | |
| | 1-1 | 2-1 | | 1-1 | 2-1 | |
| SS | 20 (19.4) | 3 (3.6) | 23 | 99 (99.2) | 8 (7.7) | 107 |
| FS | 4 (5.0) | 2 (0.94) | 6 | 43 (43.6) | 4 (3.4) | 47 |
| FF | - | - | - | 4 (3.7) | - | 4 |
| SF1 | 1 (0.84) | - | 1 | 3 (2.7) | - | 3 |
| FF1 | - | - | - | 2 (1.9) | - | 2 |
| SS1 | 2 (1.6) | - | 2 | 3 (2.7) | - | 3 |
| TOTAL | 27 | 5 | 32 | 154 | 12 | 166 |

Non-chromosome 6 markers - Serum proteins

Group Specific Component (Gc)

The data obtained for this series are shown in table 3.2.5. On examination of the phenotype distribution, there is an increase of Gc 2-2 phenotypes by 11.8% and a decrease of Gc 2-1 by 6% among the IDDM series as compared to controls. Correspondingly the gene frequencies show an increase by 8.8% in the $Gc^2$ allele. These differences, however, are not significant.

There is a tendency of slight increase in the Gc 2-1 and 2-2 phenotypes among the NIDDM and MIDDM series. But more interesting is the increase in Gc 1-1 by 7% and 9% respectively, when compared to controls. These differences are not significant in either series independently, but when combined as NIDDM+MIDDM, and then tested for heterogeneity of phenotypes, it is marginally significant. For heterogeneity of gene frequencies it is significant at less than the 2 per cent level.

Since the disturbance seen in the distribution of phenotypes and alleles in the Melbourne NIDDM and MIDDM series is also noted in the West Australian NIDDM and MIDDM combined series, it suggests that the Gc groups, which are the expression of genetically controlled variants of one of the α-globulins, are of importance in relation to susceptibility to mature-onset diabetes in the Australian population.

Haptoglobin (Hp)

Unlike the Melbourne series, there is an increase of 6.4% in Hp 1-1 and a 10% decrease of Hp 2-1 phenotypes in the MIDDM as

Table 3.2.5

Distribution of Group Specific Component (Gc) phenotypes and gene frequencies in diabetes and control population of Western Australia

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $Gc^1$ | $Gc^2$ |
|---|---|---|---|---|---|---|
| Control | 168 | 148 | 42 | 358 | 0.6760 | 0.3240 |
| IDDM | 14 | 12 | 8 | 34 | 0.5882 | 0.4120 |
| NIDDM | 38 | 29 | 4 | 71 | 0.7394 | 0.2610 |
| MIDDM | 29 | 20 | 2 | 51 | 0.7647 | 0.2353 |

Heterogeneity of phenotypes in IDDM vs. controls:

$$\chi^2_2 = 4.57 \quad P<0.10$$

gene frequency in IDDM vs. controls:

$$\chi^2_1 = 1.70 \quad P>0.20$$

phenotypes in NIDDM+MIDDM vs. controls:

$$\chi^2_2 = 5.52 \quad 0.10<P<0.05$$

phenotypes in MIDDM vs. controls:

$$\chi^2_2 = 3.70 \quad P<0.20$$

gene frequency in NIDDM+MIDDM vs. controls:

$$\chi^2_1 = 4.39 \quad 0.05<P<0.02$$

NIDDM vs. control $\chi^2_1 = 2.83$ $P>0.1$

compared to controls. The increase of Hp 1-1, however, is consistent in all three W.A. diabetic series, being 1.8%, 0.7% and 6.4% in the I, NI and MIDDM series respectively. Although these results support the observations of Berg *et al*, (1967), who also showed an increase of Hp 1-1 among the diabetic series, the distribution of Hp phenotypes and gene frequencies in the three W.A. series do not deviate significantly from that in controls (table 3.2.6).

Transferrin (Tf)

The distribution of Tf variant phenotypes is shown in table 3.2.7. There is an increase of 2.9%, 0.8% and 3.2% of the $CB_2$ phenotype in the IDDM, NIDDM and MIDDM series, with a decrease of 2.5%, 1.3% and 6% in the CC phenotype respectively. When tested for heterogeneity the differences are not significant. A decrease of 1.6%, 0.6% and 3.1% in $Tf^C$ allele frequency and an increase of 1.8%, 0.4% and 1.6% is noted in the $Tf^{B2}$ allele frequency is noted in the IDDM, NIDDM and MIDDM series respectively.

Non-chromosome 6 markers: Red cell enzymes

Esterase D

The results in table 3.2.8 for the phenotype and gene frequency distribution in the W.A. series shows for MIDDM an increase of EsT-D 2-1 phenotypes by 8.4% compared to controls; and an absence of 2-2 phenotypes compared to 2.6% in controls, but these differences are not statistically significant. The NIDDM compares well with controls. No significant deviation is seen in the IDDM series for the phenotypes and gene frequency

Table 3.2.6

Distribution of haptoglobin (Hp) phenotypes and gene frequencies in diabetes and controls of Western Australia

| Series | Phenotypes | | | No. Tested | Gene Frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $Hp^1$ | $Hp^2$ |
| Control | 52 | 172 | 149 | 373 | 0.3709 | 0.6300 |
| IDDM | 6 | 16 | 16 | 38 | 0.3684 | 0.6320 |
| NIDDM | 18 | 58 | 47 | 123 | 0.3821 | 0.6208 |
| MIDDM | 13 | 23 | 28 | 64 | 0.3828 | 0.6201 |

Heterogeneity of phenotypes in IDDM vs. controls:

$$\chi^2_2 = 1.33 \quad P=0.5$$

MIDDM vs. controls:

$$\chi^2_2 = 3.08 \quad P<0.2$$

NIDDM+MIDDM vs. controls:

$$\chi^2_2 = 0.69 \quad n.s.$$

Table 3.2.7

Distribution of transferrin (Tf) phenotypes and gene frequencies in a control and diabetic population of Western Australia

| Series | Phenotypes | | | No. Tested | Gene frequency | | |
|---|---|---|---|---|---|---|---|
| | CC | $CB_2$ | CD | | $\underline{Tf}^C$ | $\underline{Tf}^{B2}$ | $\underline{Tf}^D$ |
| Control | 361 | 6 | 1 | 368 | 0.9904 | 0.0082 | 0.0014 |
| IDDM | 21 | 1 | - | 22 | 0.9737 | 0.0263 | - |
| NIDDM | 117 | 3 | 1 | 121 | 0.9836 | 0.0123 | 0.0041 |
| MIDDM | 57 | 3 | 2 | 62 | 0.9597 | 0.0242 | 0.0161 |

Heterogeneity of gene frequency in NIDDM vs. controls:

CC vs. $CB_2$ $\chi^2_1 = 0.44$ n.s.

MIDDM vs. controls:

C vs. $B_2$ $\chi^2_1 = 3.20$ $0.05 > P > 0.1$

Table 3.2.8.

Distribution of Esterase-D (EsT-D) phenotypes and gene frequencies in a diabetic and control population of Western Australia

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency EsT-D$^1$ | EsT-D$^2$ |
|---|---|---|---|---|---|---|
| Control | 290 | 79 | 10 | 379 | 0.8694 | 0.1306 |
| IDDM | 29 | 9 | 1 | 39 | 0.8590 | 0.1410 |
| NIDDM | 96 | 26 | 3 | 125 | 0.8720 | 0.1280 |
| MIDDM | 46 | 19 | 0 | 65 | 0.8538 | 0.1462 |

Heterogeneity of gene frequency in IDDM vs. controls:

$\chi^2_1 = 0.076$ n.s.

NIDDM vs. controls:

$\chi^2_1 = 0.268$ n.s.

NIDDM+MIDDM vs. controls:

$\chi^2_1 = 0.231$ n.s.

distributions.

Acid phosphatase (PHs)

The distribution of phenotypes and gene frequencies in the control and diabetic series for acid phosphatases is given in table 3.2.9a. A decrease of 4.2% in the CB phenotypes is noted among the IDDM series, when compared to controls and an increase of 8% in the AB heterozygotes.

To examine if the decrease of CB was specific in any particular group of individuals, the data was broken into 2 categories, male and female. The numbers in the two categories are very small but the decrease of CB is noted in both sexes. In females the CB phenotype is absent while only one individual with CB is noted in the male IDDM series. In Melbourne, CB was absent among the male IDDMs. However, the data show the trend in one direction, that of a decrease in CB phenotypes in insulin-dependent diabetics. This can also be noted for the CA phenotypes in the IDDM series of this population (table 3.2.9b).

The allele frequency of $PHs^C$ in the IDDM series correspondingly shows a decrease by 3.8% when compared to controls and the NIDDM series. $PHs^A$ is decreased by 4.9% in the MIDDM series.

Although none of the above differences show statistical significance, the decrease in the phenotypes controlled by the $PHs^C$ allele in the two IDDM series (Melbourne and W.A.) is of interest and further data should be collected to confirm whether this trend is maintained.

Table 3.2.9a

Distribution of acid phosphatase (PHs) phenotypes and gene frequency in the diabetic and control population of Western Australia

| Series | Phenotypes | | | | | No. Tested | Gene frequency | | |
|---|---|---|---|---|---|---|---|---|---|
| | AA | AB | BB | CA | CB | | $PHs^A$ | $PHs^B$ | $PHs^C$ |
| Controls | 46 | 154 | 135 | 22 | 25 | 382 | 0.3508 | 0.5877 | 0.0615 |
| IDDM | 4 | 21 | 16 | 1 | 1 | 43 | 0.3488 | 0.6280 | 0.0233 |
| NIDDM | 16 | 49 | 49 | 3 | 7 | 124 | 0.3387 | 0.6210 | 0.0403 |
| MIDDM | 4 | 27 | 24 | 3 | 5 | 63 | 0.3016 | 0.6349 | 0.0635 |

Heterogeneity of gene frequency IDDM vs. controls: A+B vs. C $\chi^2_1 = 2.1$ $P<0.1$

NIDDM vs. controls: A+B vs. C $\chi^2_1 = 2.6$ $P<0.1$

MIDDM vs. controls: A+B vs. C $\chi^2_1 = 0.1$ n.s.

Table 3.2.9b.

Distribution of acid phosphatase phenotypes and gene frequencies in the male and female diabetic and control population of Western Australia

| | Phenotypes | | | | | | Gene frequency | | |
|---|---|---|---|---|---|---|---|---|---|
| Males[§] | AA | AB | BB | CA | CB | TOTAL | $PHs^A$ | $PHs^B$ | $PHs^C$ |
| Controls | 12 | 56 | 42 | 2 | 11 | 123 | 0.3333 | 0.6138 | 0.1028 |
| IDDM | 1 | 15 | 10 | 0 | 1 | 27 | 0.3148 | 0.6667 | 0.0190 |
| Females[†] | | | | | | | | | |
| Controls | 7 | 29 | 24 | 6 | 2 | 68 | 0.3603 | 0.5808 | 0.1008 |
| IDDM | 3 | 6 | 6 | 1 | 0 | 16 | 0.4063 | 0.5625 | 0.0313 |

[§] Heterogeneity of gene frequency in IDDM vs. control: A vs. B+C $\chi^2_1=0.10$ n.s.

[†] IDDM vs. control: A vs. B+C $\chi^2_1=0.16$ n.s.

Phosphoglucomutase PGM I and II

There was no variation observed at the $PGM_2$ locus in this series.

Table 3.2.10 shows the distribution of $PGM_1$ phenotypes and gene frequency in the controls and diabetic series.

As compared to controls, there is a decrease in PGM 2-1 phenotypes of 5.4% in the IDDM series and an increase of 3.4% in the MIDDM series, while the NIDDM compares well with controls. None of the differences noted are significant. An age effect was observed in the Melbourne NIDDM and MIDDM series, with a decrease of PGM 2-2 phenotypes in the older ages compared to controls. Such a trend is not present in the W.A. series. In Melbourne 25.9% of the mature-onset series are in the age range 70 and over; in the W.A. series only 11% are in this range and, therefore, this may explain the lack of a relationship between PGM and mature-onset diabetes in the W.A. series.

Glutamic pyruvic transaminase (GPT)

Table 3.2.11 shows the phenotypes and gene frequency distribution for the GPT enzyme. In the IDDM patients there is a non-significant decrease of 7.5% in the GPT 2-1 phenotype. For the NIDDM and MIDDM series, however, as shown in the Melbourne series, there is an increase of 2.6% and 13.8% in the GPT 2-2. This difference in the excess of 2-2 phenotypes does not reach significance in the MIDDM vs. controls but when the gene frequency comparison with control is made it is significant at less than the 2 per cent level. Therefore the importance of another non-chromosome 6 marker is demonstrated in the Melbourne and W.A. mature-onset

Table 3.2.10.

Distribution of phosphoglucomutase 1 ($PGM_1$) phenotypes and gene frequencies in diabetics and control population of Western Australia

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $\underline{PGM}_1^1$ | $\underline{PGM}_1^2$ |
|---|---|---|---|---|---|---|
| Control | 221 | 128 | 31 | 380 | 0.7500 | 0.2500 |
| IDDM | 25 | 11 | 3 | 39 | 0.7821 | 0.2179 |
| NIDDM | 75 | 40 | 10 | 125 | 0.7600 | 0.2400 |
| MIDDM | 36 | 24 | 5 | 65 | 0.7385 | 0.2615 |

Heterogeneity of phenotypes in IDDM vs. controls:

$$\chi^2_2 = 0.577 \quad \text{n.s.}$$

gene frequency in IDDM vs. controls:

$$\chi^2_1 = 0.306 \quad \text{n.s.}$$

phenotypes in NIDDM+MIDDM vs. controls:

$$\chi^2_2 = 0.240 \quad \text{n.s.}$$

Table 3.2.11

Distribution of glutamic pyruvic transaminase (GPT) phenotypes and gene frequencies in diabetics and control series of Western Australia

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $\underline{GPT}^1$ | $\underline{GPT}^2$ |
|---|---|---|---|---|---|---|
| Control | 63 | 89 | 37 | 189 | 0.5688 | 0.4312 |
| IDDM | 14 | 17 | 12 | 43 | 0.5233 | 0.4770 |
| NIDDM | 31 | 50 | 23 | 104 | 0.5384 | 0.4620 |
| MIDDM | 10 | 22 | 16 | 48 | 0.4375 | 0.5625 |

Heterogeneity of phenotypes in IDDM vs. controls:

$\chi^2_2 = 2.00$ $P<0.30$

MIDDM vs. controls:

$\chi^2_2 = 4.96$ $P>0.10$

NIDDM vs. controls:

$\chi^2_2 = 0.48$

gene frequency in MIDDM vs. controls:

$\chi^2_1 = 5.30$ $P<0.02$

NIDDM+MIDDM vs. controls:

$\chi^2_1 = 2.30$ $P<0.10$

NIDDM vs. control $\chi^2_1 = 0.48$ n.s.

diabetic series.

Other systems

These include 6 Phosphogluconate dehydrogenase (6PGD), adenosine deaminase (ADA) and adenylate kinase (AK). No significant variation in any of the diabetic series were observed in the phenotypes or gene frequency distributions. The data for the above systems are given in tables 3.2.12; 3.2.13; 3.2.14 respectively.

Multivariate analysis

As illustrated in figures 3.2.1 a and b, for the W.A. series, when subjected to a multivariate analysis of differences between the disease groups vs. controls, using information from 13 polymorphic systems, dispersion of the groups, to some extent, resembles the Melbourne series. The IDDM series is distant to the control and the mature-onset diabetics. However, the two mature-onset diabetic groups are not widely separated as in the Melbourne series, but are closer together. This may reflect differences between the populations of the two localities, and will be taken up for further discussion in a later section.

SUMMARY

The results obtained for the polymorphism in the W.A. population demonstrates yet again the strong association of Bf factors with the IDDM series. There is also an association of C`2 complement component although in W.A. it does not reach the level of statistical significance but it should certainly encourage the

Figure 3·2·1a

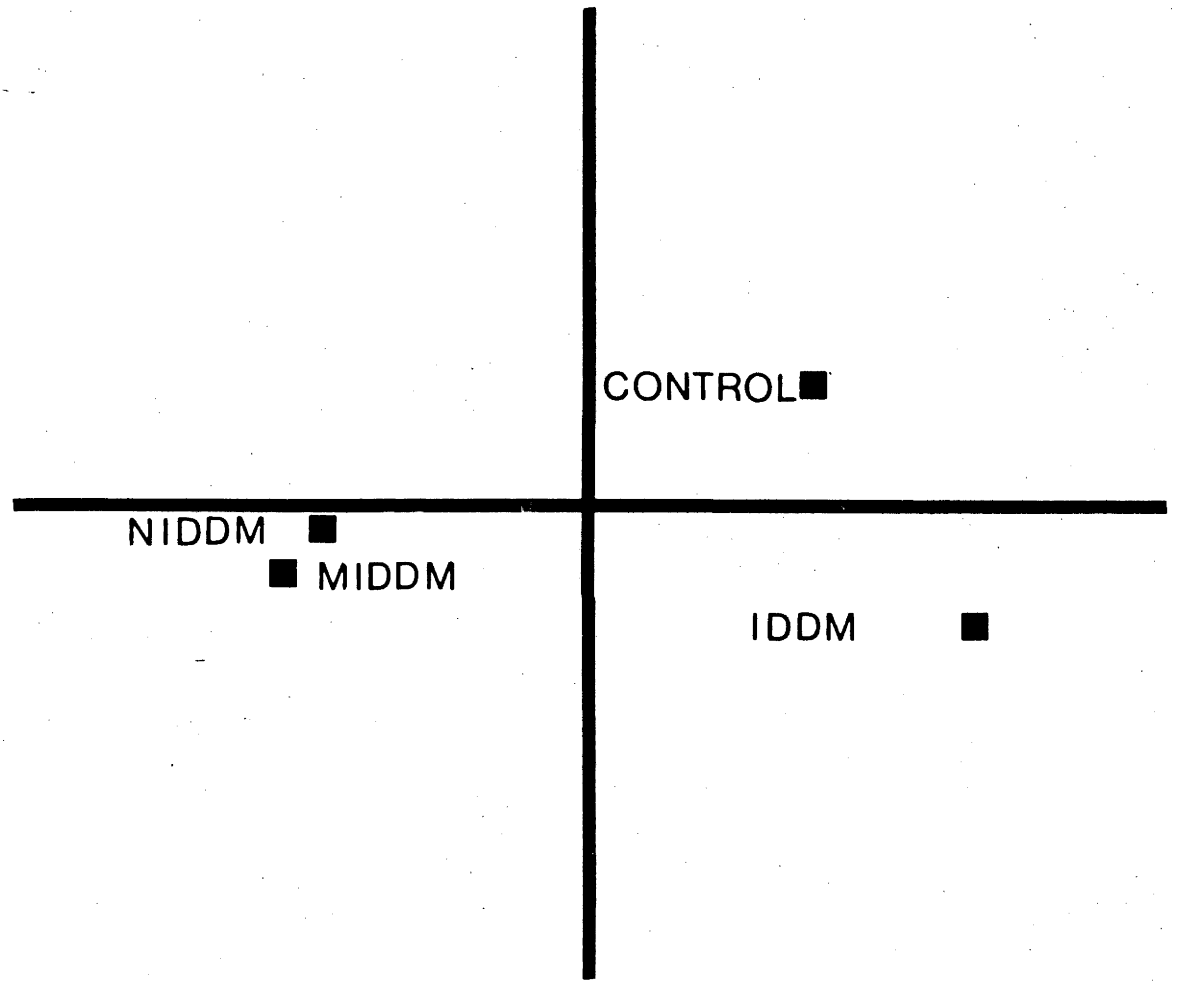



Fig. 3.2.1a. Eigenvector representation of the differences between the patients and control groups in a Western Australian population.

Figure 3·2·1b

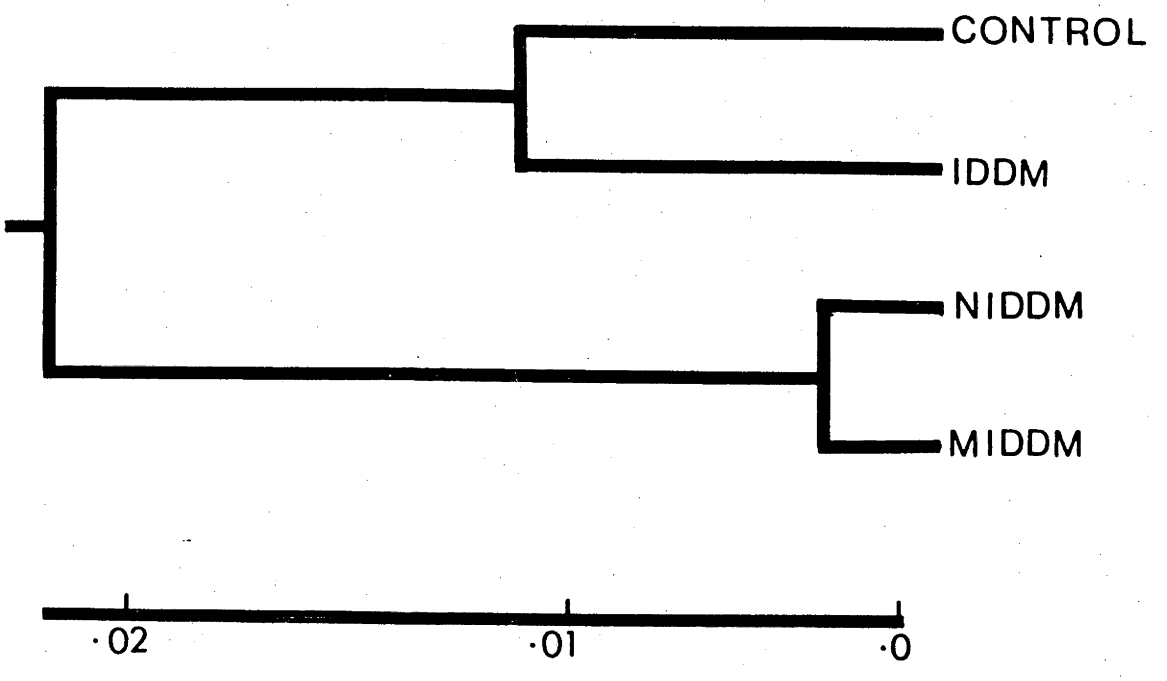



Fig. 3.2.1b. Differences between diabetic and control groups, represented by a dendrogram in a Western Australian population.

Table 3.2.12

Distribution of 6-phosphogluconate dehydrogenase (6PGD) phenotypes and gene frequencies in a diabetic and control population of Western Australia

| Series | Phenotypes | | | No. Tested | Gene Frequency | |
|---|---|---|---|---|---|---|
| | AA | AC | CC | | $\underline{6PGD}^A$ | $\underline{6PGD}^C$ |
| Control | 366 | 16 | 0 | 382 | 0.9791 | 0.0209 |
| IDDM | 39 | 0 | 0 | 39 | 1.0000 | - |
| NIDDM | 122 | 3 | 0 | 125 | 0.9880 | 0.0120 |
| MIDDM | 64 | 1 | 0 | 65 | 0.9923 | 0.0077 |

Heterogeneity of gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 0.86 \quad \text{n.s.}$$

NIDDM+MIDDM vs. controls:

$$\chi^2_1 = 1.75 \quad P>0.2$$

Table 3.2.13

Distribution of adenylate kinase (AK) phenotypes and gene frequencies in a diabetic and control population of Western Australia

| Series | Phenotypes | | | No. Tested | Gene Frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{AK}^1$ | $\underline{AK}^2$ |
| Controls | 353 | 26 | 1 | 380 | 0.9632 | 0.0368 |
| IDDM | 39 | 0 | 0 | 39 | 1.0000 | 0.0000 |
| NIDDM | 114 | 10 | 0 | 124 | 0.9597 | 0.0403 |
| MIDDM | 61 | 4 | 0 | 65 | 0.9692 | 0.0310 |

Heterogeneity of gene frequency in NIDDM+MIDDM vs. controls:

$$\chi^2_1 = 0.06 \quad \text{n.s.}$$

Table 3.2.14

Distribution of adenosine deaminase (ADA) phenotypes and gene frequencies in a diabetic and control population of Western Australia

| Series | Phenotypes | | | No. Tested | Gene Frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $ADA^1$ | $ADA^2$ |
| Control | 347 | 34 | 0 | 381 | 0.9554 | 0.0446 |
| IDDM | 34 | 4 | 0 | 38 | 0.9474 | 0.0526 |
| NIDDM | 113 | 12 | 0 | 125 | 0.9520 | 0.0480 |
| MIDDM | 52 | 12 | 0 | 64 | 0.9063 | 0.0937 |

Heterogeneity of gene frequency in IDDM vs. controls:

$$\chi^2_1 = 0.14 \quad \text{n.s.}$$

NIDDM+MIDDM vs. controls:

$$\chi^2_1 = 2.70 \quad P=0.1 \text{ n.s.}$$

collection of further data. Glyoxalase, on the other hand, does not contribute to the differences between the disease groups.

The distribution of Gc and GPT phenotypes and gene frequencies in the W.A., as in the Melbourne series, indicate the importance of other non-chromosome 6 markers which may be associated with mature-onset diabetes in patients of European origin. However, no consistent differences could be demonstrated between the NIDDM and MIDDM series, which were examined separately in this study.

A further point of interest is the decrease of the $Bf^F$ allele in both the NIDDM and MIDDM series in W.A. and in Melbourne but it is statistically significant only in W.A. Since no HLA markers are known to be associated with mature-onset diabetes, the disturbance at a closely linked locus, Properdin B, in mature-onset diabetes, is of great interest. It suggests a possible association of a chromosome 6 marker with at least some forms of NIDDM.

Chapter 4

## INDIAN POPULATION

Diabetes among the Indian population is known to vary according to diet, social stratification as well as different ethnic groups (Moses and Kannan 1976), but it is not clear whether these differences are determined by genetic or environmental factors (Ahuja 1976). A characteristic type of pancreatic diabetes is known to occur, particularly in South India, in those with very low socioeconomic and nutritional status, this is particularly true in the 16-30 age group (Viswanathan 1980).

In this age group, the frequency of diabetes is much lower than that in the European population and in several Indian series of diabetes, it is less than 1 per cent in the 16 years age group (Viswanathan *et al*, 1966; Patel and Talwalker1966). A characteristic feature in the lean population of Tropics is the resistance to ketosis despite withdrawal of insulin (Ahuja *et al*, 1965; Tripathy *et al*, 1976).

For the present investigation, however, although the details are not disclosed the samples were derived from patients attending the diabetes clinic at the All India Institute of Medical Research, New Delhi, and were kindly supplied by Professor Ahuja (details in Chapter 2). The division into disease categories includes two types based on the clinical diagnosis.

1. (IDDM) Insulin dependent diabetes, aged below 40 years.
2. (NIDDM) Non-insulin dependent diabetes aged 40 and above (including only 3 individuals who were below 40, but diagnosed NIDDM).

Since all the mature-onset patients were non-insulin diabetics,

there was no third category of MIDDM patients in the Indian population under study. In addition, samples from 50 non-diabetic individuals in New Delhi were used as controls.

Chromosome 6 markers

Glyoxalase I (GLO)

The data are given in table 4.1. The distribution of phenotypes and gene frequencies among the control and IDDM series compare well with the published figures for other unaffected Indians of New Delhi (Ghosh 1977).

In the NIDDM series, however, there is an increase of 16% in the GLO 2-2 phenotypes. This difference is not statistically significant, and can be readily explained when the composition of the patient population is examined. There is a higher proportion of residents of Punjab city (31 of 47) among the NIDDM series and 18 of the 31 are homozygous GLO 2-2 phenotypes. This is in comparison to (2 of 12) in controls. This distribution of 2-2 phenotypes is unusual and may be why there is an excess in the NIDDM series when compared to the controls. The increase of 4.8% and 10.8% in the $GLO^2$ allele in the IDDM and NIDDM respectively is not significant.

Complement factor: Properdin factor B (Bf)

The distribution of the Bf phenotypes among diabetic and controls is shown in table 4.2. The frequency of the $Bf^S$ allele in the IDDM series is increased by 5.4% and decreased by 4.7% in the NIDDM series. This is unlike the Australian series where

Table 4.1

Distribution of glyoxalase (GLO) phenotypes and gene frequency in the diabetic and control population of Indians from New Delhi

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{GLO}^1$ | $\underline{GLO}^2$ |
| Control | 4 | 16 | 13 | 33 | 0.3636 | 0.6364 |
| IDDM | 1 | 10 | 8 | 19 | 0.3160 | 0.6842 |
| NIDDM | 3 | 18 | 26 | 47 | 0.2603 | 0.7446 |

Heterogeneity of phenotypes in NIDDM vs. controls:

$$\chi^2_2 = 2.24 \quad P<0.30$$

gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 2.20 \quad P<0.10$$

Table 4.2

Distribution of properdin factor B(Bf) phenotypes and gene frequencies in the diabetic and control population of Indians from New Delhi

| Series | Phenotypes | | | | | | No. Tested | Gene frequency | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | SS | FS | FF | $SS_1$ | $S_1F$ | $F_1F$ | | $\underline{Bf}^S$ | $\underline{Bf}^F$ | $\underline{Bf}^{S1}$ | $\underline{Bf}^{F1}$ |
| Control | 20 | 18 | 5 | 1 | 2 | 1 | 47 | 0.6277 | 0.3191 | 0.0319 | 0.0213 |
| IDDM | 11 | 5 | 1 | 3 | 2 | 0 | 22 | 0.6818 | 0.2050 | 0.1136 | 0.0000 |
| NIDDM | 14 | 19 | 7 | 5 | 0 | 0 | 45 | 0.5805 | 0.3636 | 0.0568 | 0.0000 |

Heterogeneity of gene frequency in IDDM vs. controls: $\underline{Bf}^F$ $\chi^2_1 = 1.70$ $P<0.1$

$\underline{Bf}^{F1+S1}$ $\chi^2_1 = 1.80$ $P<0.1$

NIDDM vs. controls: $\underline{Bf}^F$ $\chi^2_1 = 0.75$ n.s.

$Bf^{F1+S1}$ $\chi^2_1 = 0.02$ n.s.

IDDM vs. NIDDM $\underline{Bf}^F$ $\chi^2_1 = 3.80$ $P = 0.05$

$\underline{Bf}^{S}$ frequency was increased in all the diabetic series. The numbers tested, however, are small and the differences non-significant.

Studies from this department (unpublished) and those of Mauff *et al* (1976) on the South African Indians show an absence of $\underline{Bf}^{F1}$ in that population. In the present Indian series, while there is one $BfFF_1$ individual among the controls, $F_1$ is absent in the IDDM and NIDDM series.

There is a decrease of $\underline{Bf}^{F}$ allele by 11.4% in the same series. The heterogeneity of distribution of alleles in the IDDM and NIDDM series reaches significance at the 5 per cent level but does not show significance when compared to controls. Of further interest is the increase of $\underline{Bf}^{F}$ allele among the NIDDM individuals. In this point the Indian series do not resemble either of the localities of the Australian series, where a decrease of $\underline{Bf}^{F}$ allele was noted. However, the increase of $\underline{Bf}^{F}$ in the Indian NIDDM is also non-significant.

Although Indians appear to have a very low frequency of the $\underline{Bf}^{F1}$ allele, the other rare allele, $\underline{Bf}^{S1}$, is increased in frequency compared to frequencies reported for European populations where the gene frequency approximates 1.0%. In the present study $Bf^{S1}$ has a frequency of 3.2% among controls but is 11.4% in the IDDM patients. It is increased slightly in the NIDDM series where it is 5.7%.

Unfortunately, the number of patients and controls studied is small, but the results do suggest that $\underline{Bf}^{S1}$ might be of significance as a marker among Indian IDDM patients in the same way that $\underline{Bf}^{F1}$ is a marker for IDDM in patients of European origin.

Further study of this is needed.

Complement Component C'2

Insufficient serum was available for this test to be carried out.

Non-chromosome 6 markers - Serum proteins

Group Specific Component (Gc)

Table 4.3 shows the distribution of phenotypes and gene frequencies in the control and diabetic series of N. India. Large differences in the distribution of phenotypes are observed, both in the IDDM and NIDDM series, due to the small numbers examined. These differences show an increase of Gc 1-1 and Gc 2-1 in both diabetic series. An increase of $Gc^1$ in NIDDM is similar to that of the Melbourne and W.A. series, but it is not statistically significant.

Haptoglobin (Hp)

As discussed in the earlier chapter (3.1) reports of disturbances in the phenotypic distribution of haptoglobin were noted with interest in view of the hypothesis of there being an effect of selection on the Hp 1-1 types.

In the IDDM series (table 4.4) an increase of 1.4% and 1.3% in the Hp 2-1 and Hp 2-2 are noted, whereas, in the NIDDM series a decrease of 10.4% and an increase of 6.3% are noted respectively in the above phenotypes. This compares well with the Melbourne series where similar observations were made. However, the differ-

Table 4.3

Distribution of group specific (Gc) proteins phenotypes and gene frequencies in the diabetic and control population of Indians from New Delhi

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{Gc}^1$ | $\underline{Gc}^2$ |
| Control | 18 | 14 | 4 | 36 | 0.6944 | 0.3056 |
| IDDM | 11 | 2 | 2 | 15 | 0.8000 | 0.2000 |
| NIDDM | 17 | 8 | 3 | 28 | 0.7500 | 0.2500 |

Heterogeneity of gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 0.52 \quad \text{n.s.}$$

Table 4.4

Distribution of haptoglobin (Hp) phenotypes and gene frequency in the diabetic and control population of Indians from New Delhi

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{Hp}^1$ | $\underline{Hp}^2$ |
| Control | 1 | 16 | 31 | 48 | 0.1895 | 0.8125 |
| IDDM | 0 | 8 | 15 | 23 | 0.1740 | 0.8260 |
| NIDDM | 3 | 11 | 34 | 48 | 0.1770 | 0.8230 |

Heterogeneity of phenotypes in IDDM vs. controls:

$$\chi^2_2 = 0.49 \quad \text{n.s.}$$

NIDDM+IDDM vs. controls:

$$\chi^2_2 = 0.79 \quad \text{n.s.}$$

ences are non-significant.

A range of Hp O frequency within 0.6% to 6.8% is known to occur among the general population of India (Kirk 1968), but in this series none were present in the controls and only two, one in each diabetic series, were noted.

Transferrin (Tf)

A total of only 3 transferrin variants were observed in all the persons tested. There was no deviation in the rare variants in the phenotype or gene frequency distribution in the diabetic and control series. Data are given in table 4.5.

Non-chromosome 6 markers: Red cell enzymes

Esterase-D (EsT-D)

Table 4.6 shows the phenotypes and gene frequency distributions in diabetics and controls. In a small sample of 25 IDDM patients, there were no EsT-D 2-2 phenotypes, compared to 10% in NIDDM and 6% in the control series. A corresponding decrease in the EsT-$D^2$ allele frequency of 17% and 13% is also seen in comparison to control and NIDDM respectively. This is marginally significant in the latter comparison. The difference of 4% in EsT-$D^2$ between controls and NIDDM is non-significant.

Acid Phosphatase (PHs)

The observations for this system are shown in table 4.7. In the IDDM series, there is a decrease in the phenotypic frewuency of PHs BB and PHs CB by 12% and 4% respectively while

Table 4.5

Distribution of transferrin (Tf) phenotypes and gene frequency in the diabetic and control population of Indians from New Delhi

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | CC | $CB_2$ | CD | | $\underline{Tf}^C$ | $\underline{Tf}^B$ |
| Control | 49 | 1 | - | 50 | 0.9900 | 0.0100 |
| IDDM | 25 | - | - | 25 | 1.0000 | 0.0000 |
| NIDDM | 48 | 2 | - | 50 | 0.9800 | 0.0200 |

Heterogeneity of gene frequency in NIDDM vs. controls:

$$\chi^2_2 = 0.50 \quad \text{n.s.}$$

Table 4.6

Distribution of Esterase-D (EsD) phenotypes and gene frequency in the diabetic and control population of Indians from New Delhi

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{EsT\text{-}D}^1$ | $\underline{EsT\text{-}D}^2$ |
| Control | 32 | 15 | 3 | 50 | 0.7900 | 0.2900 |
| IDDM | 19 | 6 | 0 | 25 | 0.8800 | 0.1200 |
| NIDDM | 30 | 15 | 5 | 50 | 0.7500 | 0.2500 |

Heterogeneity of gene frequency in IDDM vs. control:

$$\chi^2_1 = 1.83 \quad P>0.2$$

IDDM vs. NIDDM

$$\chi^2_1 = 3.20 \quad 0.10<P<0.05$$

Table 4.7

Distribution of acid phosphtase (PHs) phenotypes and gene frequency in the diabetic and control population of Indians from New Delhi

| Series | Phenotypes | | | | | No. Tested | Gene frequency | | |
|---|---|---|---|---|---|---|---|---|---|
| | AA | AB | BB | CA | CB | | $PHs^A$ | $PHs^B$ | $PHs^C$ |
| Control | 2 | 20 | 22 | 0 | 6 | 50 | 0.2400 | 0.7000 | 0.0600 |
| IDDM | 3 | 12 | 8 | 0 | 2 | 25 | 0.3600 | 0.6000 | 0.0400 |
| NIDDM | 7 | 20 | 23 | 0 | 0 | 50 | 0.3400 | 0.6600 | 0.0000 |

Heterogeneity of gene frequency in IDDM vs. control A vs. B+C: $\chi^2_1=2.3$ $P<0.1$

NIDDM vs. control A vs. B+C: $\chi^1_1=2.4$ $P<0.1$

an absence of PHs CB is noted in the NIDDM series, with a 10% increase of PHs AA. As pointed out for the Australian series, there is a trend of decrease in the CB phenotype in IDDM and NIDDM (W.A.) series. This is also true for the Indian IDDM and NIDDM series. The analysis on the basis of sex revealed both individuals with the CB phenotype to be present in the male IDDM series as compared to 5 female individuals with CB phenotypes in the control series.

The frequency of allele $\underline{PHs}^{A}$ is increased by 12% in the IDDM series, and that of $\underline{PHs}^{C}$ by 2%. There is a similar 10% increase of $\underline{PHs}^{A}$ and 6% of $\underline{PHs}^{C}$ in the NIDDM series.

All the above observations, although interesting, do not reach statistical significance and are therefore only suggestive of the influence of the acid phosphatase system in this Indian diabetic series. Once again the importance of further studies must be noted.

Phosphoglucomutase PGM I and II

At the $PGM_2$ locus the distribution of phenotypes was monomorphic, both in controls and the diabetic series.

For the $PGM_1$ locus, the NIDDM series differs from the controls in the distribution of PGM 1-1 and PGM 2-1 phenotypes (table 4.8). There is an 11.2% decrease in PGM 1-1 and a 7.8% increase of PGM 2-1. The same trend is seen in the IDDM series, the differences being largely due, probably, to the small numbers examined and are not statistically significant. The corresponding decrease of the $\underline{PGM}_1^1$ allele is seen in both the IDDM and NIDDM series, the decrease being 7.4% and 7.3% respectively, and being non-significant.

Table 4.8

Distribution of phosphoglucomutase I ($PGM_1$) and gene frequency in the diabetic and control population of New Delhi Indians

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{PGM}^1$ | $\underline{PGM}^2$ |
| Control | 21 | 13 | 4 | 38 | 0.7237 | 0.2763 |
| IDDM | 8 | 10 | 2 | 20 | 0.6500 | 0.3500 |
| NIDDM | 21 | 20 | 7 | 48 | 0.6508 | 0.3541 |

Heterogeneity of gene frequency in NIDDM vs. controls:

$\chi^2_1 = 1.36$ n.s.

IDDM vs. controls:

$\chi^2_1 = 0.72$ n.s.

The effect of increasing age, showing a decrease in PGM 2-2, which was noted in the Melbourne series, is not observed in the Indian diabetic series. However, on examination of the age range in this population only 10% of the NIDDM individuals are in 60-65 years of age with none over 65 years, while the remaining individuals lie in the 38-59 age group. This may account for the lack of age effect in this series when compared to the much older age structure in the mature-onset cases in Australia.

Other systems

In this series, the results obtained for ADA and GPT were not included in the analysis due to the unreliability of the samples.

For the following systems, adenylate kinase (AK) and 6-phosphogluconate dehydrogenase (6PGD) there was no significant difference in the phenotypes or gene frequencies among the diabetic and control series. The data is given in tables 4.9 and 4.10.

## SUMMARY

Of the chromosome 6 markers, the distribution of the phenotypes and allele frequency at the glyoxalase locus, does not yield informative results in contributing to the differences between the control and diabetic series.

There is an apparent association with $BfS_1$ factor in the IDDM series, suggesting its importance as a marker for this

Table 4.9

Distribution of adenylate kinase (AK) phenotypes and gene frequency in the diabetic and control population of Indians from New Delhi

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{AK}^1$ | $\underline{AK}^2$ |
| Control | 35 | 4 | 4 | 43 | 0.8604 | 0.1405 |
| IDDM | 18 | 1 | 2 | 21 | 0.8809 | 0.1200 |
| NIDDM | 41 | 7 | 2 | 48 | 0.9300 | 0.1150 |

Heterogeneity of gene frequency in NIDDM vs. controls:

$\chi^2_1 = 0.38$ n.s.

IDDM vs. controls:

$\chi^2_1 = 0.29$ n.s.

Table 4.10

Distribution of 6-phosphogluconate dehydrogenase (PGD) and gene frequency in the diabetic and control population of Indians from New Delhi

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | AA | AC | CC | | $\underline{6PGD}^A$ | $\underline{6PGD}^C$ |
| Control | 50 | - | - | 50 | 1.0000 | - |
| IDDM | 25 | - | - | 25 | 1.0000 | - |
| NIDDM | 48 | 2 | - | 50 | 0.9800 | 0.0200 |

Chi-square test was not applied due to small numbers

population. The absence of an association between IDDM and $BfF_1$ in India is not surprising, since it seems that the $BfF_1$ factor is absent or at least very rare in the normal Indian population.

Among the non-chromosome 6 markers, the trend of the distribution for the $\underline{Gc}^2$ and $\underline{PHs}^C$ noted in the Australian series, is also in the same direction.

Chapter 5

# PACIFIC ISLAND POPULATIONS

## Section 1: Western Samoa

Extensive studies on the prevalence of diabetes in Pacific Island populations are being carried out by various workers. Recent interest has stemmed from the increased prevalence rates of diabetics among many of these populations (see Chapter 1 for a summary).

Zimmet and Kirk (1979) have shown a prevalence of 10.1% and 3.6% among the urban and rural Samoans respectively. Samples from the same urban and rural Samoan population have formed the basis for the present study. Persons with a 2 hour glucose tolerance level of 160 mg% or higher were classified as diabetic. Controls were selected by matching, wherever possible, for village, age and sex from among those persons with 2 hour plasma glucose levels below 160 mg%.

Unlike Caucasians, diabetes only of the NIDDM type is seen in the Samoans, IDDM being rare or absent. The present study therefore refers only to the NIDDM type of diabetes.

### Chromosome 6 markers

#### Glyoxalase I ((GLO)

The data in table 5.1.1 show the distribution of glyoxalase 1 in the control and diabetic Samoan series. There is a 10% decrease in the GLO 2-2 phenotype in the NIDDM compared to controls with a corresponding 4.8% decrease in the $GLO^2$ allele frequency. This deviation is opposite to that seen in the mature-

Table 5.1.1

Distribution of GLO phenotypes and gene frequencies in the diabetic and control population of W. Samoa

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{GLO}^1$ | $\underline{GLO}^2$ |
| Control | 1 | 27 | 82 | 110 | 0.1318 | 0.8682 |
| NIDDM | 2 | 37 | 75 | 114 | 0.1798 | 0.8202 |

Heterogeneity of gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 2.56 \quad P<0.1$$

onset patients in both Australian series and the Indian series. In the present instance, however, the difference is statistically non-significant.

Complement factor : Properdin B(Bf)

The results are given in table 5.1.2. There is a marked decrease of the homozygous BfF phenotypes in the NIDDM series, reflected in a 5.2% decrease in the frequency of $Bf^F$. Although this is statistically non-significant it is in the same direction as the two Australian NIDDM series. There was a non-significant increase in $Bf^F$ in the Indian NIDDM series.

Of note is the presence of 2 $SF_1$ and 1 $SS_1$ persons among the NIDDM patients in Samoa. No rare Bf types were present among the Samoan controls and, indeed, $BfF_1$ and $BfS_1$ have been found only very infrequently in Pacific populations (unpublished observations from this laboratory). Since these three individuals with $BfF_1$ or $S_1$ were present exclusively in the NIDDM series, it does suggest that these types may be of importance in a small proportion of cases.

Complement component C'2

It was not possible to obtain satisfactory typing in this system for the controls series. Table 5.1.3 therefore gives results only for the NIDDM series. It is of interest only in showing that the 2-1 phenotype is present in 5% of these persons, but it is not possible to compare this with a suitable non-affected group.

Table 5.1.2

Distribution of properdin B (Bf) phenotypes and gene frequency in the diabetic and control population of W. Samoa

| Series | Phenotypes | | | | | No. Tested | Gene frequencies | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SS | FS | FF | $SF_1$ | $SS_1$ | | $\underline{Bf}^S$ | $\underline{Bf}^F$ | $\underline{Bf}^{F1}$ | $\underline{Bf}^{S1}$ |
| Control | 63 | 42 | 18 | 0 | 0 | 123 | 0.6829 | 0.3171 | 0000 | 0000 |
| NIDDM | 56 | 51 | 5 | 2 | 1 | 115 | 0.7217 | 0.2652 | 0.0087 | 0.0043 |

Heterogeneity of gene frequency in NIDDM vs. controls: $\underline{Bf}^F$ vs. S+rare $\chi^2_1 = 1.6$ P=0.20

Table 5.1.3

Distribution of C´2 phenotypes and gene frequency in a NIDDM population of W. Samoa

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{C´2}^1$ | $\underline{C´2}^2$ |
| NIDDM | 102 | 5 | 0 | 107 | 0.9770 | 0.0233 |

## Non-chromosome 6 markers - Serum proteins

### Group Specific Component (Gc)

There is no significant difference in the distribution of either phenotype or gene frequencies between NIDDM and controls (table 5.1.4). The differences which do exist, however, are in the opposite sense from those found in the Australian and Indian series, the Samoan NIDDM series having a slightly decreased $Gc^1$ frequency.

### Haptoglobin (Hp)

There is a 7% increase in Hp 1-1 phenotypes in the NIDDM series (table 5.1.5) and this is similar to the increase found in both the Australian and Indian NIDDM series. None of the differences are statistically significant. This is true also for the slight increase in the Samoan NIDDM $Hp^1$ allele frequency.

### Transferrin (Tf)

In the total Samoan series only 2 transferrin variants were found, one CD and one $CB_2$. There was no difference between the NIDDM and control series.

## Non-chromosome 6 markers: Red cell enzymes

### Esterase D (EsT-D)

Table 5.1.6 shows there is a slight decrease in the EsD 1-1 phenotypes among the NIDDM cases with a 1.4% decrease in the $EsD^1$ allele frequency. These differences are statistically non-

Table 5.1.4

Distribution of group specific component (Gc) phenotypes and gene frequency in the diabetic and control population of W. Samoa

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene frequency $\underline{Gc}^1$ | $\underline{Gc}^2$ |
|---|---|---|---|---|---|---|
| Control | 60 | 75 | 5 | 140 | 0.6964 | 0.3036 |
| NIDDM | 43 | 49 | 8 | 100 | 0.6750 | 0.3250 |

Heterogeneity of phenotypes in NIDDM vs. controls:

$$\chi^2_2 = 2.68 \quad P<0.30$$

gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 0.26 \quad \text{n.s.}$$

Table 5.1.5

Distribution of haptoglobin (Hp) phenotypes and gene frequency in the control and diabetic population of W. Samoa

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene frequency $\underline{Hp}^1$ | $\underline{Hp}^2$ |
|---|---|---|---|---|---|---|
| Control | 41 | 62 | 22 | 125 | 0.5760 | 0.4240 |
| NIDDM | 42 | 46 | 21 | 109 | 0.5963 | 0.4037 |

Heterogeneity of phenotypes in NIDDM vs. controls:

$$\chi^2_2 = 1.29 \quad P<0.50$$

gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 0.19 \quad \text{n.s.}$$

significant.

Acid phosphatase (PHs)

The $PHs^C$ allele does not occur normally in Pacific Island populations except in persons with part-European ancestry. This may explain the solitary person with the CB type among the controls (table 5.1.7).

Among the Samoan NIDDM cases there is a decrease of phenotype AA, but the decrease of the $PHs^A$ allele frequency is only marginal (1.2%). A decrease of $PHs^A$ was found also among NIDDM patients in both the Melbourne and W.A. series.

Phosphoglucomutase (PGM) I and II

No variation at PGM II locus was noted in control and diabetic series. As shown in table 5.1.8 there is a decrease of PGM 2-1 and 2-2 phenotype frequencies among the Samoan NIDDM cases compared to the controls and there is a corresponding decrease of 7.2% in the $PGM_1^2$ allele frequency, this difference approaching significance at the 5% level. The decrease in $PGM_1^2$ is significant in the Melbourne NIDDM series among persons of north European origin and this appears to be due to the decreased frequency of $PGM_1^2$ in old persons. It is of interest, therefore, that a similar trend is present in Samoans, even though only 7% and 8% respectively of the control and NIDDM series were aged 70 years and over.

Other systems

No genetic variation is found for AK in Pacific Island populations and this was true for Samoans. In addition, typing of the Samoan samples was found to be unreliable for the ADA and

Table 5.1.6

Distribution of esterase-D (Est-D) phenotypes and gene frequency in the diabetic and control population of W. Samoa

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | Est-D$^1$ | Est-D$^2$ |
| Control | 39 | 55 | 22 | 116 | 0.5733 | 0.4267 |
| NIDDM | 31 | 60 | 18 | 109 | 0.5596 | 0.4404 |

Heterogeneity of phenotypes in NIDDM vs. controls:

$$\chi^2_2 = 1.32 \quad P<0.2$$

gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 0.14 \quad \text{n.s.}$$

Table 5.1.7

Distribution of acid phosphatase (PHs) phenotypes and gene frequency in the diabetic and control population of W. Samoa

| Series | Phenotypes | | | | No. Tested | Gene frequency | | |
|---|---|---|---|---|---|---|---|---|
| | AA | AB | BB | CB | | PHs$^A$ | PHs$^B$ | PHs$^C$ |
| Control | 8 | 34 | 77 | 1 | 120 | 0.2083 | 0.7875 | 0.0042 |
| NIDDM | 1 | 45 | 74 | 0 | 120 | 0.1958 | 0.8042 | 0.0000 |

Heterogeneity of gene frequency in NIDDM vs. controls: A vs. B+C

$$\chi^2_2 = 0.11 \quad \text{n.s.}$$

Table 5.1.8

Distribution of phosphoglucomutase I ($PGM_1$) phenotype and gene frequency in the diabetic and control population of W. Samoa

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{PGM}_1^1$ | $\underline{PGM}_1^2$ |
| Control | 57 | 42 | 14 | 113 | 0.6903 | 0.3097 |
| NIDDM | 73 | 34 | 11 | 118 | 0.7627 | 0.2373 |

Heterogeneity of phenotypes in NIDDM vs. controls:

$$\chi^2_2 = 3.10 \quad P>0.25$$

gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 3.08 \quad 0.10<P<0.05$$

Table 5.1.9

Distribution of 6-phosphogluconate dehydrogenase (6PGD) phenotypes and gene frequency in the diabetic and control population of W. Samoa

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | AA | AC | CC | | $\underline{PGD}^A$ | $\underline{PGD}^C$ |
| Control | 80 | 23 | 2 | 105 | 0.8714 | 0.1286 |
| NIDDM | 88 | 28 | 4 | 120 | 0.8500 | 0.1500 |

Heterogeneity of gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 0.40 \quad \text{n.s.}$$

GPT systems and the results have not been included in the present study.

6PGD (see table 5.1.9) shows very similar phenotype distributions in the NIDDM cases and controls. There is a slight decrease of 2% in the $\underline{PGD}^{A}$ allele frequency in the NIDDM series, but the difference is non-significant.

## SUMMARY

The Samoan NIDDM series show similarity with the NIDDM series of Australia at two loci, one on chromosome 6 (Bf) and the other a non-chromosome 6 marker (PGM). At the Properdin factor B locus, there is no significant increase of Bf rare alleles but a decrease of the $\underline{Bf}^{F}$ is noted in the NIDDM series. The trend for GLO 2 phenotypes is, however, in the opposite direction. At the non-chromosome 6 markers there is an absence of $\underline{PHs}^{C}$ and a non-significant decrease of $\underline{PGM}^{2}$ allele is noted in the NIDDM series and the trend for $\underline{Gc}^{2}$ is again in the opposite direction to the Melbourne series. At the other loci no significant disturbance in the phenotype or gene frequencies were noted.

# Chapter 5

## Section 2: Nauru

The highest known prevalence of diabetes in the Pacific Island population is that of 42% among the Micronesians of Nauru (Zimmet and Kirk, 1979). The age and sex specific incidence shows a higher incidence among the males 53.5%, compared to females 48.2% at ages 45-54 years. Based on a recent study, Zimmet and Kirk (1979) suggest a genetic difference in the Micronesian population when compared with Polynesians with respect to the significantly higher plasma insulin concentration in the former.

The Nauru population's experience of diabetes compares closely with the prevalence among the Pima Indians. There is a marked similarity in the occurrence of a bimodal curve for the distribution of two hour blood glucose values in the two populations and the two components enable the separation into NIDDM and normal individuals, unlike the unimodal distribution among Caucasians. IDDM type diabetes is absent, or of rare occurrence in Nauru.

This study includes individuals in the first and second components of the bimodal curve. The criteria used for diagnosis are outlined in Chapter 2.

Due to improper conditions of storage of the red cells and serum samples, results of certain enzymes (GLO and ADA) and complement C´2 were not reliable and are therefore excluded from the discussion.

Chromosome 6 markers

Complement factor; Properdin factor B (Bf)

The data obtained for this population is shown in table 5.2.1. In contrast to the previous observations of other populations, there is an absence of homozygotes Bf FF, both in the control and the NIDDM series. The frequency of Bf SS is higher than that in the Australian series in controls, decreasing by 5% in the NIDDM series. The rare types, infrequent among Pacific populations are absent in Nauruans. There is a non-significant increase of $Bf^F$ by 2.1% in the NIDDM series, which is similar to the Indian series, but not with the trend observed in the Australian and Samoan series.

Non-chromosome 6 markers - Serum proteins

Group Specific Protein (Gc)

The results of the observations of the Nauru population are given in table 5.2.2. The phenotypic distribution in the NIDDM series, like the Australian and Indian series, shows a tendency for a decrease in the Gc 2-2 phenotype, which is reflected in the slight decrease of $Gc^2$ allele frequency. This difference is, however, not significant.

Haptoglobin (Hp)

An increase of Hp 2-1 and 2-2 is noted in the NIDDM series (table 5.2.3), reflected by an increase of 5.2% in the $Hp^2$ allele frequency. This trend is similar to that followed in the

Table 5.2.1

Distribution of Properdin factor B (Bf) phenotypes and gene frequencies in the diabetic and control population of Nauru

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | SS | FS | FF | | $\underline{Bf}^S$ | $\underline{Bf}^F$ |
| Control | 88 | 15 | - | 103 | 0.9301 | 0.0781 |
| NIDDM | 65 | 16 | - | 81 | 0.9012 | 0.0990 |

Heterogeneity of phenotypes in control vs. NIDDM patients:

$$\chi^2_1 = 0.92 \quad \text{n.s.}$$

gene frequency in control vs. NIDDM patients:

$$\chi^2_1 = 1.00 \quad P = 0.3$$

Table 5.2.2

Distribution of group specific component (Gc) phenotypes and gene frequencies in the diabetic and control population of Nauru

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{Gc}^1$ | $\underline{Gc}^2$ |
| Control | 57 | 40 | 6 | 103 | 0.7476 | 0.2524 |
| NIDDM | 51 | 25 | 7 | 83 | 0.7650 | 0.2350 |

Heterogeneity of phenotypes in NIDDM vs. controls:

$$\chi^2_2 = 1.70 \quad \text{n.s.}$$

gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 0.16 \quad \text{n.s.}$$

Table 5.2.3

Distribution of haptoglobin (Hp) phenotypes and gene frequencies in the diabetic and control population of Nauru

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{Hp}^1$ | $\underline{Hp}^2$ |
| Control | 22 | 47 | 33 | 102 | 0.4461 | 0.5539 |
| NIDDM | 12 | 39 | 29 | 80 | 0.3940 | 0.6063 |

Heterogeneity of phenotypes in control vs. IDDM patients:

$$\chi^2_2 = 1.4 \quad P = 0.50$$

gene frequency in control vs. NIDDM patients:

$$\chi^2_1 = 1.8 \quad P>0.2$$

Table 5.2.4

Distribution of Esterase D (EsD) phenotypes and gene frequencies in the diabetic and control population of Nauru

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{EsT\text{-}D}^1$ | $\underline{EsT\text{-}D}^2$ |
| Control | 25 | 54 | 30 | 109 | 0.4770 | 0.5230 |
| NIDDM | 41 | 60 | 44 | 145 | 0.4906 | 0.5104 |

Heterogeneity of phenotypes in NIDDM vs. controls:

$$\chi^2_2 = 1.8 \quad \text{n.s.}$$

gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 0.03 \quad \text{n.s.}$$

Melbourne, Indian and Samoan series. The difference in this series is not statistically significant.

Transferrin (Tf)

There is a very high frequency of $Tf^C$ allele among the Pacific populations and this is also true for the Nauru control and NIDDM series. No rare variants were observed in this study.

Non-chromosome 6 markers: Red cell enzymes

Esterase-D

Unlike the low frequency of EsT-D 2-2 phenotypes among the Australian and Indian series, EsT-D 2-2 is high in Pacific populations as shown for example with the Samoan series. This is also true of the NIDDM series in the Nauru populations (table 5.2.4). There are no significant differences between the control and NIDDM in the phenotypes or gene frequency distribution.

Acid Phosphatase (PHs)

Acid phosphatase has two alleles, $PHs^A$ and $PHs^B$, in the control and NIDDM series of Nauru (table 5.2.5). There is an absence of $PHs^C$ allele, as noted also in the other Pacific population of Samoa (with one exception). The trend of a decrease of the $PHs^A$ allele is seen in the NIDDM series and also compares with that in the Melbourne and Samoan series. This is, however, not statistically significant.

Table 5.2.5

Distribution of acid phosphatase (PHs) phenotypes and gene frequencies in the diabetic and control population of Nauru

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | AA | AB | BB | | $\underline{PHs}^A$ | $\underline{PHs}^B$ |
| Control | 8 | 44 | 57 | 109 | 0.2752 | 0.7250 |
| NIDDM | 11 | 51 | 83 | 145 | 0.2517 | 0.7483 |

Heterogeneity of phenotypes in NIDDM vs. controls:

$\chi^2_2 = 0.74$ n.s.

gene frequency in NIDDM vs. controls:

$\chi^2_1 = 0.37$ n.s.

Table 5.2.6

Distribution of phosphoglucomutase (PGM) phenotypes and gene frequencies in the diabetic and control population of Nauru

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{PGM}^1_1$ | $\underline{PGM}^2_1$ |
| Control | 87 | 21 | 1 | 109 | 0.8945 | 0.1055 |
| NIDDM | 105 | 36 | 4 | 145 | 0.8483 | 0.1517 |

Heterogeneity of gene frequency in NIDDM vs. controls:

$\chi^2_1 = 2.5$ $P<0.10$

Phosphoglucomutase (PGM) I and II

Like the other populations, the distribution of $PGM_2$ is monomorphic also in the population of Nauru.

An increase of 5.6% and 1.9% is noted in PGM 2-1 and $PGM_1$ 2-2, reflected in a non-significant increase of 4.6% of the $PGM^2$ allele (table 5.2.6). In this respect, the observation compares with the Indian NIDDM series but shows an opposite trend to the Australian and Samoan series.

An examination of the age composition shows individuals over 70 years of age to comprise only 3.3% and 1.7% of the NIDDM and control series of Nauru, which is a much lower percentage than that seen in the Australian NIDDM series. The majority in the Nauru series belong to the 25-60 age group. Therefore the correlation with higher age noted in the Australian series does not occur in this population.

6-Phosphogluconate dehydrogenase (6PGD)

The data in table 5.2.7 shows the distribution of 6PGD phenotypes and gene frequencies in the control and NIDDM series of the Nauru population.

Interestingly, in the NIDDM series, there is an excess of heterozygote 6PGD AC types, being 6.5% higher than that in controls. Only 2 homozygotes 6PGD CC are observed compared to none in controls. The allele frequency $6PGD^C$ is consequently increased by 4.6% and this increase is highly significant at less than the 0.1% level ($\chi^2_1 = 7.8$ $0.01<P<0.001$).

It is of interest to note that reports of association with variants of 6PGD are known for some infectious diseases, like

Table 5.2.7

Distribution of 6-phosphogluconate dehydrogenase (6PGD) phenotypes and gene frequencies in the diabetic and control population of Nauru

| Series | Phenotypes | | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|---|
| | AA | AC | CC | | $6PGD^A$ | $6PGD^C$ |
| Control | 107 | 2 | 0 | 109 | 0.9908 | 0.0092 |
| NIDDM | 131 | 12 | 2 | 145 | 0.9448 | 0.0552 |

Heterogeneity of gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 7.78 \quad 0.01<P<0.001$$

Individuals with $\underline{6PGD^C}$: RR = 2.41

the tuberculoid form of leprosy, where 6PGD$^{C}$ and 6PGD$^{Wantoat}$ were increased in patients with the above form of the disease (Serjeantson *et al*, 1979). Also in patients over 41 years with hepatomegaly and splenomegaly, 6PGD AC was reported to be in excess (Harrison *et al*, 1976). Both the above observations were made on populations in New Guinea.

The possible association with PGD$^{C}$ in the NIDDM in the Nauru diabetic population is intriguing . Due to low frequency of 6PGD$^{C}$ in other populations the direction towards an increase or decrease cannot be associated. The association may, therefore, be exclusively for some forms of NIDDM in the Nauru population. This study encourages the need for further data to confirm this phenomenon.

## SUMMARY

The restricted number of genetic markers examined in the Nauru series show several differences of distribution compared with the Australian NIDDM series. This is true particularly for the Bf and PGM polymorphism in which it resembles the distribution among the Indian NIDDM patients. Similarity with Samoans is noted in the absence of the PHs$^{C}$ allele

Of some significance is the possible association of 6PGD$^{C}$ exclusively in the NIDDM series of Nauru. It may contribute as a marker, not on chromosome 6, for some forms of NIDDM in this population. However, conclusions cannot be drawn until further data to confirm the above are examined.

Chapter 6

## PIMA INDIAN POPULATION

The American Indians of Arizona have been the focus of considerable epidemiologic study during recent years. The Pima population has, to date, the highest prevalence of diabetes in the world: 50% for those over 35 years of age(Bennett *et al*,1971).

As pointed out in the previous chapter, there are two features in common between Nauruans and Pima Indians; 1. the bimodal population distribution of the frequency of blood glucose concentration after glucose load which separates individuals into normal and diabetic and 2. the IDDM type of diabetes is rare or absent. The criteria used for diagnosis is outlined in Chapter 2. Controls were selected from individuals with 2 hour plasma glucose levels below 200 mg%.

Among those diagnosed as diabetic some were treated by oral tablets and, in some more severe cases, insulin was administered. Due to the difficulties in identification of individuals by the form of treatment on the records made available, all the patients were regarded as a single category in the NIDDM series.

It is worthy of note that only four genetic markers have been examined previously in the Pima Indians. These are two of the red cell enzymes, Glyoxalase I and Phosphoglycolate phosphatase (Cadien *et al*, 1979) and two serum proteins, haptoglobin and transferrin (Matson *et al*, 1968; Kirk, 1971).

Chromosome 6 markers

Glyoxalase I (GLO)

The distribution of phenotypes and gene frequencies for glyoxalase is given in table 6.1. As can be observed the data for the NIDDM series are almost identical with the controls. The trend of an increase of the GLO 2-2 phenotype, noted in the Australian and Indian series, therefore, is not seen among the Pima Indians. The possible reasons for these discrepancies between the various populations will be discussed more fully in the final chapter of this thesis.

Complement factor ; Properdin factor B(Bf).

The Bf system is almost invariant among the Pima Indians. A single FS individual was noted among the controls and two FS and one $SS_1$ persons in the diabetic series (table 6.2). From the information provided on ethnic origin, the single individual with the Bf $SS_1$ phenotype was found to be admixed with other tribes with known European admixture. This may be a possible explanation for the occurrence of the solitary $SS_1$ type. Because of the very low frequency of variants in the Pimas, Bf is unlikely to be of any value as a genetic marker for diabetes in this population.

Complement component C'2

The rare occurrence of C'2 2-2 phenotypes in Europeans is also true for the Pima Indians (table 6.3) and the per cent phenotypic distribution compares closely with the distribution in the

Table 6.1

Distribution of glyoxalase (GLO) phenotypes and gene frequency in the diabetic and control population of Pima Indians

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $GLO^1$ | $GLO^2$ |
|---|---|---|---|---|---|---|
| Control | 11 | 59 | 71 | 141 | 0.2872 | 0.7130 |
| NIDDM | 8 | 46 | 55 | 109 | 0.2844 | 0.7206 |

Heterogeneity of phenotypes in NIDDM vs. controls:

$$\chi^2_2 = 0.020 \quad \text{n.s.}$$

gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 0.005 \quad \text{n.s.}$$

Table 6.2

Distribution of properdin factor B (Bf) phenotypes and gene frequency in the diabetic and control population of Pima Indians

| Series | Phenotypes SS | FS | FF | $SS_1$ | No. Tested | Gene Frequency $Bf^S$ | $Bf^F$ | $Bf^{S_1}$ |
|---|---|---|---|---|---|---|---|---|
| Control | 141 | 1 | - | - | 142 | 0.9965 | 0.0035 | 0000 |
| NIDDM | 106 | 2 | - | 1 | 109 | 0.9862 | 0.0092 | 0.0050 |

Due to the very small numbers $\chi^2$ test was not applied.

Melbourne series. In this series there is an increase of 3.8% of C´2 2-1 in the NIDDM series, unlike the decrease noted in the C´2 2-1 among the NIDDM of Melbourne series.

The heterogeneity of phenotypes in the control vs. NIDDM of Pima Indians is not statistically significant. The $C´2^2$ allele frequency is correspondingly increased by 2% and again is not statistically significant. More data on this system may be of interest among the NIDDM series of Pima Indians.

Non-chromosome 6 markers - Serum proteins

Group Specific Protein (Gc)

The distribution of phenotypes and gene frequency for Gc among the controls and NIDDM is given in table 6.4. There is no significant difference between the NIDDM series and controls. It may be noted, however, that the trend of an increase in $Gc^2$ noted in the Samoans is also present in the Pima NIDDM series.

Haptoglobin (Hp)

As shown in table 6.5, the distribution of haptoglobin phenotypes and gene frequencies in the NIDDM series, compare very well with controls. No trend of an increase in the $Hp^2$ noted in the earlier populations, can be seen in the NIDDM series of Pima Indians.

Transferrin (Tf)

The fast variant $CB_1$ is distributed with almost the same frequency in the NIDDM as in controls, and in the controls two

Table 6.3.

Distribution of complement component C´2 phenotypes and gene frequency in a control and diabetic population of Pima Indians

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency C´2 | C´2 |
|---|---|---|---|---|---|---|
| Control | 135 | 6 | - | 141 | 0.9790 | 0.0213 |
| NIDDM | 101 | 9 | - | 110 | 0.9591 | 0.0410 |

Heterogeneity of gene frequency NIDDM vs. controls:

$$\chi^2_1 = 1.3 \quad \text{n.s.}$$

phenotypes NIDDM vs. controls:

$$\chi^2_2 = 2.0 \quad \text{n.s.}$$

Table 6.4

Distribution of group specific component (Gc) phenotypes and gene frequency in a diabetic and control population of Pima Indians

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $\underline{Gc}^1$ | $\underline{Gc}^2$ |
|---|---|---|---|---|---|---|
| Control | 113 | 25 | 3 | 141 | 0.8901 | 0.1109 |
| NIDDM | 85 | 21 | 3 | 109 | 0.8761 | 0.1239 |

Heterogeneity of gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 0.31 \quad \text{n.s.}$$

Table 6.5

Distribution of haptoglobin (Hp) phenotypes and gene frequency in a diabetic and control population of Pima Indians

| Series | Phenotypes | | | No. Tested | Gene Frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | $\underline{Hp}^1$ | $\underline{Hp}^2$ |
| Control | 43 | 68 | 28 | 139 | 0.5540 | 0.4460 |
| NIDDM | 34 | 50 | 23 | 107 | 0.5514 | 0.4490 |

Heterogeneity of phenotypes in NIDDM vs. controls:

$$\chi^2_2 = 0.21 \quad \text{n.s.}$$

Table 6.6

Distribution of transferrin (Tf) phenotypes and gene frequency in a diabetic and control population of Pima Indians

| Series | Phenotypes | | No. Tested | Gene frequency | |
|---|---|---|---|---|---|
| | CC | CB | | $Tf^C$ | $Tf^B$ |
| Control | 137 | 4 | 141 | 0.9908 | 0.0142 |
| NIDDM | 105 | 4 | 109 | 0.9817 | 0.0183 |

Heterogeneity of gene frequency NIDDM vs. control:

$$\chi^2_1 = 0.33 \text{ n.s.}$$

of these individuals are admixed Pima Indians. There are no significant differences between the NIDDM and control series among the Pima Indian population (table 6.6).

## Non-chromosome 6 markers: Red cell enzymes

### Esterase D (EsT-D)

Table 6.7 gives the distribution of EsT-D phenotypes and gene frequencies. There is an increase of 7.7% in EsT-D 2-1 and a slight decrease of 2.8% in the EsT-D 2-2 phenotypes, these differences are not statistically significant.

A slight increase of $EsT\text{-}D^2$ allele in the NIDDM series maintained the trend observed in W.A. and Samoan NIDDM populations, although the difference again is non-significant.

### Acid Phosphatase (PHs)

Two alleles of the acid phosphatase occur frequently in this population (table 6.8). A decrease of the PHs AB phenotype by 8% and an increase of PHs BB of 5.3% are noted in the NIDDM series and these are statistically non-significant. However, the decrease of $PHs^A$ allele is consistent with that observed in all the other populations except the Indian NIDDM series. The $PHs^C$ allele is frequent only in Caucasoids and is a Caucasian gene. The admixture with European population is considered to be about 6-7% in the Pima population, which may explain the solitary heterozygote CA in the controls.

Table 6.7

Distribution of Esterase-D (EsT-D) phenotypes and gene frequency in a diabetic and control population of Pima Indians

| Series | Phenotypes | | | Tested | Gene Frequency | |
|---|---|---|---|---|---|---|
| | 1-1 | 2-1 | 2-2 | | EsT-D$^1$ | EsT-D$^2$ |
| Control | 76 | 57 | 8 | 141 | 0.7411 | 0.2589 |
| NIDDM | 54 | 52 | 3 | 109 | 0.7340 | 0.2660 |

Heterogeneity of phenotypes in NIDDM vs. controls:

$$\chi^2_2 = 2.10 \text{ n.s.}$$

gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 0.03 \text{ n.s.}$$

Table 6.8

Distribution of acid phosphatase (PHs) phenotypes and gene frequency in a diabetic and control population of Pima Indians

| | Phenotypes | | | | No. Tested | Gene Frequency | | |
|---|---|---|---|---|---|---|---|---|
| | AA | AB | BB | CA | | PHs$^A$ | PHs$^B$ | PHs$^C$ |
| Control | 3 | 51 | 86 | 1 | 141 | 0.2057 | 0.7907 | 0.0035 |
| NIDDM | 6 | 31 | 73 | 0 | 110 | 0.1955 | 0.8050 | 0.0000 |

Heterogeneity of gene frequency in NIDDM vs. control:

$$\chi^2_1 = 0.05 \text{ n.s.}$$

phenotypes in NIDDM vs. controls:

$$\chi^2_2 = 2.25 \text{ n.s.}$$

Phosphoglucomutase I and II (PGM)

The distribution at the $PGM_2$ locus was not polymorphic in this series of Pima Indians.

At the $PGM_1$ locus, interestingly, there is a decrease of PGM 1-1 phenotypes in the NIDDM series by 13.9% as compared to controls, and an increase of 3.1% in the PGM 2-2 types (table 6.9). This trend is the reverse of that observed in the Australian and Samoan NIDDM series, but is similar to Indian and Nauru NIDDM population, where also a decrease in PGM 1-1 was noted. The decrease in the 1-1 phenotype is reflected in the decrease of $\underline{PGM}_1^1$ allele, which is significant at a 1 per cent level.

There is a difference in the age composition of the Pima NIDDM patients when compared to the Australian NIDDM series, in that only 2 individuals over 70 years were included in the Pima patients, the large majority being in the 18-66 years range. Therefore the possible association of $\underline{PGM}_1^1$ in Pima NIDDM is seen in a series with a different age composition to that of $\underline{PGM}_1^2$ in a higher age range of the Melbourne series.

These observations, therefore, indicate the importance of a non-chromosome 6 marker in the Pima NIDDM population and also necessitate further data to confirm the above phenomenon.

Glutamic pyruvic transaminase (GPT)

Although the NIDDM series showed differences in the phenotypic distributions when compared to controls, there being an 8.5% increase of GPT 1-1 and a 14.6% decrease of GPT 2-1 (table 6.10), these differences are not statistically significant and

Table 6.9

Distribution of phosphoglucomutase I ($PGM_1$) phenotypes and gene frequency in a diabetic and control population of Pima Indians

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $\underline{PGM}_1^1$ | $\underline{PGM}_1^2$ |
|---|---|---|---|---|---|---|
| Control | 105 | 34 | 2 | 141 | 0.8652 | 0.1348 |
| NIDDM | 66 | 38 | 5 | 109 | 0.7808 | 0.2201 |

Heterogeneity of gene frequency $\chi^2_1 = 6.25$ P=0.01

RR = 1.14 Woolf's $\chi^2_1 = 0.31$ n.s.

Table 6.10

Distribution of glutamic pyruvic transaminase (GPT) phenotypes and gene frequency in a diabetic and control population of Pima Indians

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $\underline{GPT}^1$ | $\underline{GPT}^2$ |
|---|---|---|---|---|---|---|
| Control | 24 | 84 | 30 | 138 | 0.4782 | 0.5220 |
| NIDDM | 28 | 50 | 30 | 108 | 0.4907 | 0.5103 |

Heterogeneity of phenotypes NIDDM vs. controls:

$\chi^2_2 = 4.36$ P<0.1

gene frequency NIDDM vs. controls:

$\chi^2_1 = 0.13$ n.s.

may be due to the small sample sizes. Moreover, the $\underline{GPT}^2$ allele is decreased only very slightly in comparison to controls. The earlier NIDDM series in the Australian population showed this decrease to be significant.

Phosphoglycolate phosphatase (PGP)

The polymorphism in phosphoglycolate phosphatase (PGP) has been described only recently by Barker and Hopkinson (1978) and it was not included in the battery of genetic marker tests for the other populations in the present study. However, because of the recent claim by Cadien *et al* (1979) of an association between $\underline{PGP}^1$ and diabetes in the Pima Indians it was decided to test for this enzyme system in the samples available for study.

In the present investigation the data observed for the NIDDM and control series are shown in table 6.11. There is a 5.9% increase of PGP 1-1 and 4.1% decrease of PGP 2-2 phenotypes. Neither of these differences are statistically significant.

The observations made by Cadien *et al* (1979) showed a large increase of $\underline{PGP}^1$ allele frequency (24%) in the NIDDM vs. control comparison, which was significant. In the present investigation, however, although there is an increase of 5% in the $\underline{PGP}^1$ allele frequency, it does not show statistical significance. This discrepancy in significance of the two observations may well be due to the number of individuals examined: total control + NIDDM = 64 in Cadien *et al*'s study vs. control + NIDDM = 205 in the present study.

Table 6.11

Distribution of phosphoglycolate phosphate (PGP) phenotypes and gene frequency in a diabetic and control population of Pima Indians

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $PGP^1$ | $PGP^2$ |
|---|---|---|---|---|---|---|
| Control | 55 | 46 | 19 | 120 | 0.6500 | 0.3500 |
| NIDDM | 44 | 31 | 10 | 85 | 0.7000 | 0.3000 |

Heterogeneity of phenotypes in NIDDM vs. controls:

$$\chi^2_2 = 0.99 \quad \text{n.s.}$$

gene frequency in NIDDM vs. controls:

$$\chi^2_1 = 1.40 \quad \text{n.s.}$$

Table 6.12

Distribution of adenylate kinase (AK) phenotypes and gene frequency in a diabetic and control population of Pima Indians

| Series | Phenotypes 1-1 | 2-1 | 2-2 | No. Tested | Gene Frequency $AK_1$ | $AK_2$ |
|---|---|---|---|---|---|---|
| Control | 140 | 1 | - | 141 | 0.9965 | 0.0035 |
| NIDDM | 108 | 1 | - | 109 | 0.9954 | 0.0046 |

Chi-square test is not applied due to small numbers.

Other systems

The AK 2-2 phenotype is of rare occurrence among the Pima Indians both in control and the NIDDM series (table 6.12), and the $AK^1$ allele is distributed with 99.5% frequency in the Pima Indians. No variation in the NIDDM from the controls were observed.

6-Phosphogluconate dehydrogenase likewise shows a 100% distribution of the $6PGD^A$ allele and in contrast to the Nauruans, there is an absence of the $6PGD^C$ allele in both the control and NIDDM series. (The data is not shown in table form).

## SUMMARY

In this population, the chromosome 6 markers, GLO and Bf do not contribute to differences between the NIDDM series and controls. A tendency towards an increase of $C'2^2$, noted in Pima NIDDM's is unlike that found in the Australian series.

The observations for the PGP system in this study do not support the association with NIDDM claimed by other workers.

Among the other markers studied there is a significant increase in the $PGM_1^2$ allele among the Pima NIDDM patients which contrasts with a decrease of this allele in Australia and Samoan NIDDM's. No significant differences in gene or phenotypic frequencies were observed in any of the other systems studied.

Chapter 7

# DISCUSSION

The objective of this investigation has been an attempt to demonstrate how genetic markers can be used to throw further light on the complexity of diabetes mellitus. This has involved an examination of serum protein and enzyme markers under genetic control, in a series of patient and control populations. The series of diabetic patients and controls were derived from two distinct localities of Australia - Melbourne and Western Australia, and others from widely separated geographical and environmental areas provided by a series from North India, Micronesia (Nauru), Polynesia (Samoa) and from an Aboriginal population of North America.

The patient series were divided where possible into two categories: IDDM or type I - insulin dependent, aged less than 40 years at onset, and NIDDM or type II - mature-onset, aged 40 years and above. This latter group, particularly in the Australian series, was further divided into those receiving insulin (MIDDM) and those being maintained either by diet alone or diet supplemented with oral hypoglycaemic agents (NIDDM). This division into M and NIDDM types is undoubtedly arbitrary, since the form of treatment may change from that at the time of diagnosis. A patient on a treatment by diet or oral agents alone may transfer to insulin after several years, and such a sub-division, which distinguishes the duration of treatment in some patients, may be necessary in view of the heterogeneity of diabetes. Further studies should take such sub-division into

account, but large series will be required since each specific sub-division reduces the number of patients to a point where statistical significance is harder to achieve, when associations, even if real, are not strong. The problems of small numbers of patients has been troublesome in the present study and is demonstrated particularly with respect to all series except those in Australia. This makes many of the present conclusion only tentative and it will require further indepth study to either confirm or refute some of the associations reported.

The significant associations found, as well as the trends observed in each population, have been summarized in table 7.1 and the importance of these associations and trends are discussed in detail below.

The genetic markers investigated have been considered in two separate groups - those controlled by loci on chromosome 6 and those controlled by loci on other chromosomes. This distinction was made because previous studies demonstrated highly significant associations between HLA genes, controlled by at least 4 loci on chromosome 6 and the IDDM type of diabetes.

Chromosome 6 markers

This study has demonstrated a highly significant association between the IDDM type and one of the markers on chromosome 6 (Properdin B). Some details of which have been published recently (Theophilus *et al*, 1980; Kirk *et al*, 1979) and also confirmed by simultaneous studies of Raum *et al*, 1979, Deschamps *et al*, 1979; Bertrams *et al*, 1979).

In the present series the increased relative risk, particularly

Table 7.1

Comparison of the distribution of associations and trends in

| SYSTEM | AUSTRALIA | | | |
|---|---|---|---|---|
| | MELBOURNE | | W.A. | |
| | IDDM | NIDDM | IDDM | NIDDM |
| Bf$^{F1\&S1}$ | $^{**}$Increase | $S_1$ Increase<br>$F_1$ Decrease | $^{**}$Increase | θ |
| BfF | $^{**}$Decrease | Decrease | $^{**}$Decrease | $^{*}$Decrease |
| C'2$^{2}$ | $^{**}$Increase | Decrease | Increase | Decrease |
| GLO$^{2}$ | θ | $^{+}$Increase | $^{+}$Decrease | Increase |
| Gc$^{2}$ | Decrease | $^{++}$<br>$^{**}$Decrease | Increase | $^{+}$<br>$^{**}$Decrease |
| Hp$^{2}$ | Decrease | Increase | θ | Decrease |
| PHs$^{C}$ | Decrease<br>CB male | Decrease | Decrease<br>CB male | Decrease |
| PHs$^{A}$ | Increase | Decrease | Decrease | Decrease |
| PGM$^{2}$ | θ | $^{**+}$Decrease | Decrease | Decrease |
| GPT$^{2}$ | - | Increase | - | Increase |
| 6PGD$^{C}$ | Increase | θ | θ | Decrease |
| EsT-D$^{2}$ | $^{+}$Decrease | Decrease | Increase | Decrease |
| PGP$^{1}$ | - | - | - | - |

Significance of gene frequency $^{*}$ P=0.05

$^{**}$ P=0.02-0.01

phenotypic frequency $^{+}$ P=0.05

$^{++}$ P=0.02-0.01

no difference = θ

Those not done are represented by -

serum proteins and red cell enzymes in different diabetic populations.

| INDIA | | SAMOA | NAURU | PIMA |
|---|---|---|---|---|
| IDDM | NIDDM | NIDDM | NIDDM | NIDDM |
| Increase | Increase | Rare | Absent | Rare |
| Decrease | Increase | Decrease | Increase | θ |
| - | - | - | - | Increase |
| Increase | Increase | Decrease | - | Increase |
| Decrease | Decrease | Increase | Decrease | Increase |
| Increase | Increase | Decrease | Increase | θ |
| Decrease | Decrease | Absent | Absent | Absent |
| Increase | Increase | Decrease | Decrease | Decrease |
| Increase | Increase | *Decrease | Increase | **Increase |
| - | - | - | - | Decrease |
| θ | θ | Increase | **Increase | Absent |
| Decrease | Decrease | Increase | Decrease | Increase |
| - | - | - | - | Increase |

for the Bf $F_1$ factor was demonstrated first for the IDDM patients in the Melbourne series, but increased $F_1$ frequencies were present also in the Western Australian IDDM series, and in the north Indian series for the Bf $S_1$ factor. Bf $F_1$ is absent, or has only a low frequency, in north Indians so it is of interest that there is a suggestive increase in the $S_1$ frequency in the small IDDM series from Delhi. This will be discussed again.

It is important to note also that among Samoans, Nauruans and Pima Indians, Bf $F_1$ and $S_1$ are also absent or of rare occurrence and when they do occur may indicate admixture with persons of European descent. It is of significance also, perhaps, that in these populations IDDM of the classical European type is absent or of very rare occurrence.

Examination of the ages of IDDM patients in the Melbourne series showed a striking association between Bf $F_1$ and age of onset. Approximately 20% of patients aged 0-4 years had the $F_1$ factor, and this fell rapidly to the frequency of controls in older IDDM patients. MIDDM and NIDDM patients also had frequencies of Bf $F_1$ not significantly different from that of controls.

This striking association of Bf $F_1$ with younger IDDM patients raises interesting possibilities about the biological mechanism involved in this association. One such possibility is that the gene controlling the $F_1$ factor may result in a decrease in the level of properdin factor B in the serum, and it is known from animal experiments that decreased levels of properdin increase the risk of infection (Pillemer *et al*, 1954, 1955; Newman *et al*,

1978). However, the present study shows no evidence at present to support such a mechanism in relation to the involvement of properdin in an infectious aetiology of IDDM.

The more likely mechanism is that Bf is in linkage disequilibrium with IDDM susceptibility alleles, and these latter alleles are the ones involved in controlling the immune responses seen in the development of IDDM. The specific susceptibility allele(s) of significance for failure to combat an infection leading to irreversible B cell destruction in the young child appears to be in strong linkage disequilibrium with the $F_1$ factor, at least in a significant proportion of cases of IDDM in Europeans.

Further evidence to support the view that Bf factors are in linkage disequilibrium with specific immune functions in IDDM is provided by studies on lymphocytotoxic antibodies in IDDM in children. One study (Serjeantson *et al*, 1981) showed a correlation of the presence of LCA's in IDDM children with the time since onset. In IDDM patients 12 months after onset sera show 55% LCA's compared to 25% in those after one to two years and 15% in those after five years since onset of disease. In the same study it has been demonstrated that $\underline{Bf}^{F1}$ allele is in significant positive association with LCA's than in patients without this allele. Conversely, the alleles $\underline{Bf}^{F}$ and $\underline{Bf}^{S1}$ are associated with a reduced frequency of LCA's, significantly less than patients without these alleles.

When investigated for an association of LCA with HLA-B locus antigens the IDDM patients positive for HLA-B18 had a high risk for LCA's (RR 7.16). It may be noted that since $BfF_1$ and HLA-B18 are in stronger positive linkage disequilibrium in IDDM than in controls, their significant association with LCA is as expected.

However, this is not true for HLA-B8 and $BfF_1$ association, since from the data shown earlier, they are in negative disequilibrium among the IDDM patient series.

The studies of Bf were extended to the Indian population where the apparent association in IDDM is with the $BfS_1$ factor. The absence of an association between IDDM and $BfF_1$ is not surprising since as noted above the $BfF_1$ is absent, or at least very rare in normal Indian populations. For the Indian IDDM series, it would seem that $\underline{Bf}^{S1}$ may confer increased risk in IDDM, comparable to $BfF_1$ in the European series.

Although HLA studies were not performed on the north Indian series, information from other sources show a different HLA profile in India compared to European populations. One study on South African Indians (Hammond and Asmal 1980) shows a slight increase of HLA-B 40.2 in the 'Aryan' Indian IDDM patients and, unlike the European, no increase in the frequency of HLA-B8 or B15 was noted. $BfS_1$ is known to be in linkage disequilibrium with HLA-B21 in the European population but this may differ in the Indian series, a likely candidate may be B40.2, but this speculation needs further data. It would be of interest to note whether the IDDM susceptibility allele in the Indian series is in linkage disequilibrium with $\underline{Bf}^{S1}$. The importance of Bf demonstrated in the IDDM series in the population of European origin, may not be limited to type I diabetes only. This is based on the observation of a significant decrease (in the W.A. series) of $\underline{Bf}^{F}$ among the NIDDM cases. Although this trend of decrease was also present in the Melbourne and Samoan NIDDM series, it was not significant in the latter while in the Indian NIDDM series the trend was in the opposite direction.

Unfortunately, the extent of genetic variability of the Bf system is not so extensive in Pacific and American Indian population and its importance as a marker for diabetes in these populations is thereby reduced. As pointed out earlier, type I diabetes also is known to be infrequent in these populations. Whether this may be due to the absence of susceptibility alleles linked to Bf and HLA is an important question demanding further research.

The complement C´2 system also has been shown to be important in type I diabetes in Australia. This is not unexpected since C´2 is very close to the Bf locus and linkage disequilibrium is possible with Bf alleles, although not demonstrated by present data. It is also suggested that C´2 and factor B (Bf), in the classical and alternate pathway respectively, may be homologous and tandemly duplicated. This is based on the close physico-chemical similarity between the two (Lachmann and Hobart, 1978).

Since C´2 has not been investigated sufficiently in other populations to warrant any firm conclusions, but indicates again that in Pacific populations the C´2 system is relatively invariant, so that it is not likely to be of significance in those populations in relation to diabetes.

The third chromosome 6 marker, the red cell enzyme system glyoxalase, was investigated fully because it is also thought to be linked to HLA.

The present study shows some intriguing associations between GLO and NIDDM, particularly in the Australian series. GLO was not found to be significantly associated with the IDDM series in

the Australian, or with IDDM, in the Indian series, but there was a significant disturbance of the GLO 2 phenotypes and $\underline{GLO}^2$ allele in the Melbourne NIDDM series. A similar trend is non-significant in W.A. and in the Indian NIDDM series. The significant disturbance of GLO phenotypes in the Melbourne series appears to be, at least to some extent, an artifact of ethnic stratification in the sample. When the patients were subdivided on the basis of ethnic origin, into more homogenous groups, the significance of the difference was reduced although the trend of an increase of $\underline{GLO}^2$ allele remained the same. A similar situation of stratification was noted in the increase of $\underline{GLO}^2$ allele among the Indian NIDDMs although non-significant. The trend of distribution was opposite in the Samoan NIDDM series and the $\underline{GLO}^2$ frequencies in the Pima NIDDM and controls were almost identical.

One explanation which may apply for the increase of the $\underline{GLO}^2$ allele frequency, is the presence of a null allele, which has been demonstrated in the European population, and may result in the excess of GLO 2-2 phenotypes. However, the null allele at the GLO locus has very low frequency and it seems unlikely to account for the disturbance noted in the Melbourne series. A second explanation is a suggestion of selection effect upon the GLO locus, also noted by other workers (Busi *et al*, 1979). Cadien *et al*, (1979) reported an association of $\underline{GLO}^1$ with diabetes in Pacific Island populations. However, observations in the present investigation show an increase in the $\underline{GLO}^1$ allele in Samoan

diabetes but this is not significant and therefore does not support the association suggested by Cadien and his colleagues.

Non-chromosome 6 markers

The reason for studying markers linked to HLA and putative disease susceptibility alleles was clear-cut in the study of IDDM, where HLA associations had already been established. The study of non-HLA linked markers, however, requires more justification. There are three possible mechanisms which can be involved in defence of such a research strategy. (1) the locus in question controls a gene product directly involved in a pathway important for glucose metabolism; (2) that the locus is linked to another locus concerned with an immune mechanism or, alternatively, a metabolic control locus of importance in diabetes, and (3) that genetic variation at one or more loci may reveal a more general genetic differentiation of persons susceptible to diabetes from persons in the general population.

As examples of (1) it may be that several of the loci studied here may be of potential importance if their variant alleles disturbed the normal metabolism of glucose - 6PGD for example, or even glyoxalase. In the latter case GLO is not directly involved in a glucose metabolism, but it could alter equilibrium conditions of glucose metabolism indirectly. Such suggestions, however, are highly speculative and no evidence is currently available to support them.

The second mechanism is of greater significance. Experimental work with mice has revealed several histocompatibility loci separate from those in the major H2 complex. By analogy there

may be similar loci therefore in man and if enough polymorphic loci are screened it may be possible to find one or more in linkage disequilibrium with those other histocompatibility or disease susceptibility loci. Finally, if susceptibility to diabetes is under polygenic control, as is suggested by some of the analyses of diabetic families, then susceptible individuals, as a group, will have a constellation of genes linked to their multiple diabetic genes which will make them a genetically distinctive group.

The present study has focussed attention on two of these research strategies, firstly looking for consistent associations between alleles at specific loci and, secondly, examining the general genetic differentiation of diabetes from controls.

With respect to the first strategy it is important to point out that an association between a specific allele and one or other form of diabetes need not be the same in different populations. Particular linkages may be strong in one population but absent in another, as has been demonstrated clearly for the association between HLA factors and diabetes in various parts of the world and from the association with Bf rare alleles in this study.

Other workers, as noted in Chapter 1, have drawn attention to associations between the ABO blood groups and also the Lewis blood group system and diabetes. For the ABO blood goups there is a suggestive effect of age, which may be related to the type of diabetes. In the present study it was not possible, however, to obtain data on any of the red cell antigen systems.

Among the serum protein systems studied, two have previously been implicated with diabetes: the group specific component

system (a vitamin D-binding protein) and haptoglobin (a haemoglobin-binding protein) have both been reported as showing non-significant disturbances among diabetics in populations of European origin.

In four of the six populations investigated here there is a decrease in the frequency of the $\underline{Gc}^2$ allele in the NIDDM series. In two Australian series the decrease is significant and there is also a significant difference in the distribution of phenotypes between patients and controls. The increase in $\underline{Gc}^2$ frequency among NIDDM patients in Samoa and Pima Indians is non-significant. The disturbance in the Gc system is most marked in the Melbourne and W.A. series. This fact, coupled with previous reports of a similar trend in European diabetics, certainly suggests that further studies of the association between Gc and mature-onset diabetes should be undertaken urgently. This is of particular importance since the Gc system has been implicated also in susceptibility to Kuru in the New Guinea Highlands, to leprosy and also to the degree of reaction to typhoid vaccination, although Gc cannot be considered an immune-response system itself (Cleve, 1973).

Another serum protein system studied here is the haemoglobin-binding glycoprotein, haptoglobin. Variants of haptoglobin are universally polymorphic, and the greater power of the Hp 1-1 protein for removing haemoglobin from the circulation after red cell breakdown compared to the Hp 2-2 protein, has led to suggestions that the $\underline{Hp}^1$ gene is favoured by selection, at least in some situations. The present study, however, does not suggest that the haptoglobin locus is of any significance

with respect to diabetes.

No significant association with the Hp alleles were noted, nor was there any trend for an increase or decrease of $\underline{Hp}^1$ or $\underline{Hp}^2$ among diabetics in the populations studied. These findings are consistent with other studies of the haptoglobin types, particularly among the NIDDM series, in various populations. It seems that much larger sample sizes would be needed to detect the effects of selection against one or other of these alleles, if such in fact does occur.

Among the red cell enzyme loci studied the one which is of greatest interest in the present context is that controlling red cell acid phosphatase. The precise biological function of this enzyme is not clear, but its polymorphic variation in human populations makes it a valuable marker for linkage disequilibrium studies. In the present investigations there is a consistent decrease of the phenotype controlled by the $\underline{PHs}^C$ allele, the CB phenotype in particular, and this is most striking among male IDDM patients in Australia (both series) and also among IDDM in north India. Since the $\underline{PHs}^C$ allele is of rare occurrence in Pacific populations and Amerindians it is not a useful marker there, although in this respect there is a parallel between the absence of rare alleles in the Bf system in Pacific and Amerindian populations and the absence of IDDM in the same populations. The present observations support the earlier report of a decrease of the CB phenotype in an IDDM series in Rome (Lucarelli *et al*, 1978).

The situation is different in the NIDDM series. There is a decrease of $\underline{PHs}^A$ in NIDDM patients in all the localities

studied, except in north India. Despite this trend none of the differences noted were large, and none were statistically significant.

The interpretation of the apparent association between the acid phosphatase system and diabetes is difficult. It is of interest, however, that Bottini *et al* (1971) has reported an association between red cell acid phosphatase phenotypes and favism and that acid phosphatase phenotypes also appear to be affected by factors such as diet and temperature (Ananthakrishnan and Walter 1972). The association with diabetes may be therefore related to some pleiotropic effect of the genes concerned.

One of the more interesting results obtained in the present investigation involves the phosphoglucomutase system. There were decreases in the $\underline{PGM}^2$ allele frequency for NIDDM in the two Australian series and in the Melbourne series which was highly significant for persons of north European ancestry. In one further population (Samoa) there was also a significant decrease of $\underline{PGM}^2$ in NIDDM, whilst in the Pima NIDDM series there was a significant increase of $\underline{PGM}^2$. $\underline{PGM}^2$ was increased also in the north Indian and Nauruan NIDDM series, but these increases were not statistically significant.

Within Australia there is a contrast between the Melbourne and W.A. NIDDM series. The decrease of $\underline{PGM}^2$ in the former was much greater and the reason for this contrast appears to be related to age. As pointed out in Chapter 3.1, Gordon and Riser (1966) showed a decrease in $\underline{PGM}^2$ in persons aged 80 years and over. When the Melbourne and W.A. NIDDM series are compared there is a much higher proportion of older persons among the

Melbourne patients. The $\underline{PGM}^2$ frequency is lowest among those aged 80 and over, with patients in the 40-69 age group intermediate in their $\underline{PGM}^2$ frequency between the older group and the controls. The W.A. $\underline{PGM}^2$ frequency for the NIDDM series is almost identical with the $\underline{PGM}^2$ frequency for the Melbourne 40-69 group.

There are two possible interpretations for this effect. Firstly, persons with the $\underline{PGM}^2$ may not be so susceptible to developing non-insulin dependent diabetes (alternatively persons with a $\underline{PGM}^1$ allele are more susceptible). The second possibility is that persons with the $\underline{PGM}^2$ allele are less likely to survive to older ages. Since NIDDM increases in prevalence with age, the diabetic series will be drawn from a population with a decreasing $\underline{PGM}^2$ frequency as mean age of the patients increases.

An urgent study is required to discriminate between those two possibilities. PGM typing of a geriatric population divided into diabetic and non-diabetic should enable a choice to be made.

Since the PGM effect appears to be strongly age-related it would be expected that non-significant differences would be found between the NIDDM series and controls in populations without a large proportion of very old persons. This is true for north India and Nauru. The two remaining populations, Samoa and Pima Indians, however, are not consistent. The former gives a significant decrease and the latter a highly significant increase in PGM frequency among NIDDM patients. Lack of detailed information on these respective patient series has

prevented more detailed analysis. The PGM system, however, will clearly repay further study.

Four other enzyme systems showing polymorphic variation were examined in detail, GPT, 6PGD, EsT-D and PGP. With two exceptions none of the comparisons between diabetes and controls for any of the populations where tests were made, revealed significant differences. One of the exceptions was a highly significant increase in the $\underline{6PGD}^{C}$ allele among diabetics in Nauru. A similar trend of increase of $\underline{6PGD}^{C}$ was note in Samoan NIDDM cases, but in the other populations differences were small. An increase in 6PGD AC has been reported in tuberculoid leprosy in New Guinea (Serjeantson *et al*, 1979) suggesting that the 6PGD locus might be involved in susceptibility to some diseases. Confirmation of the association with leprosy is needed, and further study of the relationship between 6PGD and diabetes in Nauru is needed.

The other exception was a decrease of the Esterase-D 2-2 phenotype among IDDM patients in the Melbourne series. Since this was significant only at the 5 per cent level and 9 comparisons for Est-D were made in total, the difference could be due to sampling errors. Of possible interest is a previous by Williams and Cartwright (1978) who found also no association between Est-D and diabetes even when the diabetics were categorized on the basis of age.

The enzyme phosphoglycolate phosphatase (PGP) was studied only in Pima Indians. A paper (in abstract only ) by Cadien *et al*, 1979) appeared when the experimental work for this thesis was almost complete. Cadien and his colleagues claimed a

significant increase in the $\underline{PGP}^1$ allele among diabetic Pima Indians. The Pima series was therefore tested for PGP variants to see if their claim could be substantiated. The results of these observations did show an increase in $\underline{PGP}^1$ in Pima NIDDM compared to controls but the difference was non-significant. Since the present observations were based on a total series of 205 persons compared to 64 in Cadien *et al's* study, it seems unlikely that PGP is associated with non-insulin dependent diabetes.

One final question which needs to be addressed is whether diabetes in older persons is a heterogenous disorder ? For examination of this possibility the diabetic patients in the present study, aged 40 and over, who were receiving insulin treatment, were separated as a group (MIDDM) from those not requiring insulin (NIDDM). This distinction was possible only for the Melbourne and W.A. series but the numbers of MIDDM patients in both series were not large (81 and 65 respectively). In comparisons based on individual genetic marker systems there were no significant phenotypic or genotypic differences between MIDDM patients and controls, nor were there any significant differences between MIDDM and NIDDM patients in the same locality. The absence of statistical significance in both these sets of comparisons indicates no striking associations which would be demonstrable even with small numbers. In the next section, however, it will be pointed out that MIDDM patients do appear to be genetically distinct from IDDM and NIDDM patients.

Multivariate analysis.

The final research strategy exploited was to carry out a multivariate analysis which enables the contribution from all the variable genetic systems to be utilized to see if there are genetic differences between diabetics as a group, compared to the normal population. The technique employed was a measure of genetic 'distance', the basis of which was discussed in Chapter 2. In the present study it was applied only to the Australian series. Future studies could profitably apply similar techniques to the other series for which the data is generated.

When all markers are included in the analysis, IDDM cases clearly are differentiated from both controls and M- and NIDDM cases and this is demonstrated both in the Melbourne and W.A. series (see Figs 3.1.1a and b and 3.2.1a and b) although the distinction is more striking in the Melbourne series that for W.A. There is another contrast between the two series, however, in the distinction between the NIDDM and MIDDM patients. In Melbourne these two groups are genetically quite distinct, but in W.A. they are very close to one another, even though both groups are distinct from the control and IDDM series.

The multivariate technique, therefore, reinforces the classification of diabetes on the basis of insulin dependence in persons younger than 40 years, and this is true in two separate localities in Australia. It also suggests that a further distinction can be made genetically between mature-onset cases (40 years and over at time of onset) and controls, and, in Melbourne, between mature-onset cases receiving insulin

and those not.

The fact that this latter distinction does not hold in W.A. could well reflect differences in the structure of the patient population in these two localities. It was pointed out earlier that the Melbourne NIDDM series had a much higher frequency of persons aged 70 years and over, compared to W.A., so the two series may not be identical genetically. The discussion of differences in PGM types has made clear that such genetic differences do exist between the two populations. In addition, there may be problems of ethnic stratification reinforcing the genetic differences and possibly contributing to the variations in type and severity of the diabetes found in either Melbourne or W.A. Further study should explore the effects of such differences more fully.

Ideally patients should be matched with controls of the same age and economic status from the same ethnic group and be subjected to the same set of clinical observations and biochemical tests. Such specific divisions into clinical features is important in view of the heterogeneous nature of diabetes, and may enable the identity of types within the complex syndrome. If this can be done for an adequate number of patients a better understanding of the role of genetic markers, in relation to diabetes, certainly would be achieved.

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# Genetic Susceptibility to Diabetes Mellitus: he Distribution of Properdin Factor B (Bf) and Glyoxalase (GLO) Phenotypes

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**UMMARY**

**he distribution of phenotypes controlled by two loci n chromosome 6 has been studied in a series of 239 atients with type 1 (insulin-dependent) and 297 pa- ients with type 2 (non-insulin-dependent) diabetes ellitus. At the properdin factor B (Bf) locus there is a ignificant increase in the frequency of the *Bf*$^{S1}$ and *Bf*$^{F1}$ alleles for type 1 patients, and the combined in- rease in frequency of *Bf*$^{S1}$ and *Bf*$^{F1}$ in those patients s highly significant. The relative risk for F1 is 6.2 and or F1 and S1 combined is 5.3. These results confirm he association with F1 reported recently by Raum nd co-workers in Boston. The two rare alleles *Bf*$^{S1}$ nd *Bf*$^{F1}$ are in significant negative disequilibrium with LA B8.**

**For the glyoxalase (GLO) locus there is a slight but onsignificant increase in the frequency of the *GLO*$^{2}$ llele, but a significant disturbance in the distribution f the GLO phenotypes for type 2 patients. These re- ults for the GLO alleles may be due to stratification n our series of type 2 patients. Further studies are in rogress to test this hypothesis. DIABETES *28*:949–951, ctober 1979.**

Renewed attention has been given recently to the role of genetic factors in susceptibility to diabetes mellitus,[1–4] and this has received added impetus from the studies of the distribution of human leucocyte antigens (HLA) in diabetic patients. Several surveys[5–9] have shown a significant increase in some antigens, particularly HLA B8 and BW15 for Caucasian patients with type 1 (juvenile or insulin-dependent) diabetes mellitus, and the association is even stronger with HLA DW 3 and DW 4. Among Japanese patients with type 1 diabetes, there is an increased risk associated with HLA B12 or BW 22.[10,11] These associations with HLA antigens have not been observed for patients with type 2 (maturity-onset or non-insulin-dependent) diabetes mellitus. However, twin studies suggest that susceptibility to type 2 diabetes of the non-insulin-dependent type does have a strong genetic component.[12]

As part of the search for further associations between diabetes and other genetic markers, Raum et al.[13] recently reported a highly significant increase in the frequency of the F1 type in the properdin factor B (Bf) system in patients with juvenile diabetes. We also have similar evidence for a significant increase in the frequency of some phenotypes in the serum properdin factor B (Bf) system in patients with type 1 diabetes, and for an unusual distortion in the distribution of phenotypes in the glyoxalase (GLO) system in type 2 diabetes. Both the serum system Bf and the cell enzyme system GLO are controlled by loci close to HLA on the short arm of chromosome 6.[14,15] The locus for Bf is very close to that for HLA B, while that for GLO is between HLA D and the centromere. Cudworth et al.[1,16] previously reported an increase in *Bf*$^{S}$ for type 1 diabetes, but so far there are no reports on the distribution of GLO types in either type 1 or type 2 diabetes.

## PATIENTS AND METHODS

Blood samples were collected from patients with type 1 diabetes attending the Diabetic Clinic at the Royal Children's Hospital in Melbourne and from a further series attending the Diabetic Clinic at the Royal Southern Memorial Hospital, Melbourne. The majority of patients in the latter series were of the non-insulin-dependent type with age of onset 40 yr or over. A smaller number in this series were maturity-onset cases but were on insulin therapy, while a few cases in this older age group were type 1, fully insulin dependent. Control series of blood samples were obtained from nonselected blood donors attending the Blood Transfusion Service in Canberra and Sydney. Bf and GLO typing was performed using standard procedures.[17,18]

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TABLE 1
Bf types and gene frequencies in diabetes

| Series | No. tested | Bf phenotypes | | | | | | | | Percent gene frequencies | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S | FS | F | $S_1F$ | $SF_1$ | $SS_1$ | $FF_1$ | $F_1$ | $Bf^S$ | $Bf^F$ | $Bf^{S1}$ | $Bf^{F1}$ |
| Type 1 (IDDM) | 239 | 163 | 38 | 3 | 1 | 25 | 6 | 2 | 1 | 82.6 | 9.8 | 1.5 | 6.1 |
| Type 2 (NIDDM) | 292 | 203 | 74 | 8 | 1 | 2 | 4 | 0 | 0 | 83.2 | 15.6 | 0.9 | 0.3 |
| Controls | 380 | 248 | 100 | 20 | 1 | 5 | 3 | 3 | 0 | 79.5 | 19.0 | 0.5 | 1.0 |

## RESULTS

**Bf types.** Four Bf alleles segregated in the series studied and in the controls. Type 1 patients have a significant increase in the frequency of the rare alleles $Bf^{S1}$ and $Bf^{F1}$ when compared with the controls (for the two alleles combined $\chi^2$ for type 1 versus controls = 22.8, $P \ll 0.001$). This increase in frequency of the rare alleles is not present in the type 2 patients.

As shown in Table 1 both type 1 and type 2 patients show a small increase in the common $Bf^S$ allele when compared with our normal controls, but the differences are nonsignificant. For both type 1 and type 2 patients there is a compensatory decrease in the frequency of the other common allele, $Bf^F$. This decrease is exaggerated in type 1 patients, and the difference from controls for $Bf^F$ for these patients is highly significant ($\chi^2 = 21.5$, $P \ll 0.001$).

**GLO types.** The distribution of GLO phenotypes and the frequencies of the two GLO alleles is almost identical in patients with type 1 diabetes and in controls. For type 2 patients there is a decrease in the frequency of the $GLO^1$ allele, but the difference between the patients and controls is nonsignificant ($\chi^2 = 1.48$, $P = 0.2–0.3$). Type 2 patients, however, have an unusual distribution of GLO phenotypes. There is an excess of homozygous 2–2 individuals and a deficiency of heterozygotes compared with the number expected when the gene frequencies derived from the controls are applied to the patient series. Indeed, the type 2 series, when considered alone, is not in genetic equilibrium ($\chi^2 = 5.8$, $P = 0.02$)(Table 2).

## DISCUSSION

These results confirm and extend the finding of Raum and his colleagues of a strong association between insulin-dependent diabetes and the F1 type in the Bf system, although in our own larger series, the level of association is not so high. The Boston study reported that 22.6% of their patients carry an F1 gene compared with 1.9% of their controls. In the Australian type 1 patients we have found 11.7% with a F1 gene and 0.6% and 2.1%, respectively, in type 2 patient and controls. We have also found a slight increase in the S gene. The relative risk in type 1 patients with F1 gene in th Bf system is 6.2, and for F1 and S1 combined it is 5.3.

Since type 1 diabetes is associated with an increase i the frequency of HLA B8 and BW15, it would not be surpri ing if there was also a change in frequency of Bf alleles, b cause the loci for HLA B and Bf are very close. Indee since it has been shown[19,20] for European and Iceland populations that HLA B8 is in strong linkage disequilibriu with $Bf^S$, the frequency of $Bf^S$ should be increased in type diabetics. This was found to be true by Cudworth et al., and in the present series of type 1 patients there is a 3% i crease in the frequency of $Bf^S$. We find a similar increase $Bf^S$ frequency, however, in type 2 patients. In neither seri is this increase in the frequency of $Bf^S$ significant.

Because $Bf^{S1}$ and $Bf^{F1}$ are associated significantly wi type 1 diabetes, we have examined the linkage disequili rium between HLA and Bf in our type 1 patients. Significa disequilibrium values occurred for HLA A1 with $Bf^F$ and B with $Bf^F$. More interesting, however, is the finding that HL B8 has significant negative disequilibrium values with $Bf^S$ $Bf^{F1}$, and $Bf^F$.

Our series of type 1 patients shows the increase in HL B8 frequency (48% versus 24%)[21] found in other studies type 1 diabetes among Europeans. The negative disequili rium values between HLA B8 and $Bf^{S1}$ and $Bf^{F1}$, both which also have significantly increased frequency amon our type 1 patients, suggests that there is a diabetic susce tibility allele in linkage disequilibrium with HLA B8 in som cases and with $Bf^{S1}$ and $Bf^{F1}$ in others. The negative disequ librium between HLA B8 and $Bf^F$ probably reflects th known strong positive disequilibrium between B8 and $Bf^S$ normal European populations.

The lack of significant associations between alleles at e ther the Bf or GLO loci for type 2 patients is in agreeme with previous studies, which have failed to show associ tions between HLA types and type 2 patients. Our own r sults indicate, however, a significant disturbance in the di tribution of GLO phenotypes with an excess of 2–2 an deficiency of heterozygous 2–1 phenotypes. In the absen of evidence implicating other chromosome 6 markers type 2 diabetes, the present results may be due to stratific tion in the series investigated here. Further studies are progress to check the reproducibility of the disturbance the distribution of GLO phenotypes, with adequate contro to eliminate confounding effects.

The genetic heterogeneity of diabetes is again demo strated clearly in our studies of non-HLA loci on chrom

TABLE 2
GLO types and gene frequencies in diabetes

| Series | No. tested | GLO phenotypes | | | Percent gene frequencies | |
|---|---|---|---|---|---|---|
| | | 1-1 | 2-1 | 2-2 | $GLO^1$ | $GLO^2$ |
| Type 1 (IDDM) | 247 | 46 | 126 | 75 | 44.1 | 55.9 |
| Type 2 (NIDDM) | 292 | 58 | 121 | 113 | 40.6 | 59.4 |
| Controls | 382 | 72 | 195 | 115 | 44.4 | 55.6 |

me 6. The significant association between type 1 patients d some Bf phenotypes is not found in type 2 patients. In ntrast, alleles at the other chromosome 6 locus, glyoxa- se, show no significant difference from controls for either pe 1 or type 2 diabetics.

We are extending these studies to diabetic patients in ner parts of the Western Pacific to see if the results re- rted here are supported more generally.

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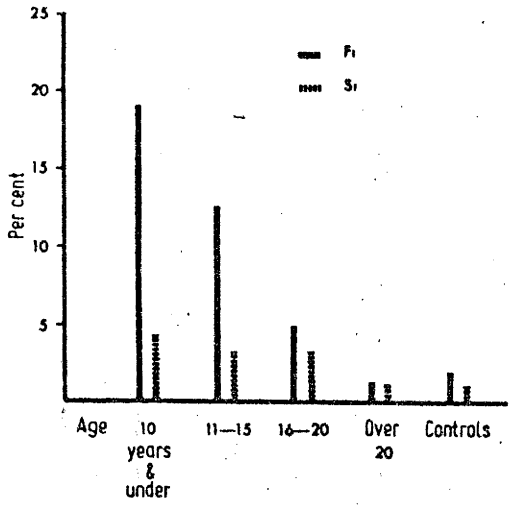


**Relationship of age to frequency of rare Bf factors in 281 Australian IDDM patients.**

## AGE RELATIONSHIP BETWEEN INSULIN-DEPENDENT DIABETES AND RARE ALLELES OF PROPERDIN FACTOR B

SIR,—The report by Raum and colleagues[1] of a striking association between insulin-dependent diabetes (IDDM) and the re genetic type F1 in the properdin factor B system has been nfirmed independently in studies from our laboratories ported to a meeting of the Human Genetics Society of Australia (Queenstown, New Zealand, June 27–29, 1979). Raum al. found the Bf F1 factor in 22·6% of U.S. White IDDM tients compared with 1·9% of the general population, giving relative risk of 15·0. In our study of 281 Australian IDDM tients 8·9% had the F1 factor compared with 2·1% in 380 ntrols, a relative risk of 4·5. Our study indicates also that re is a slight increase in the frequency of the other rare Bf ctor, S1, being 2·8% in patients and 1·1% in controls. Raum al. also reported a similar increase in S1: 2·4% *v* 1·0%

The discrepant estimates of relative risk that people with the factor will have IDDM may well reflect different age compositions of the two study populations, although Raum et al. not provide age distributions for their U.S. patients. The quency of Bf F1 is highly age-dependent (see accompanying ure). The F1 factor is present in 19·1% of 47 Australian IDM patients aged 10 years or less, falling progressively to % in 79 patients over 20 years of age. If the frequency of Bf F1 and S1 factors are combined we find a decline from ·4% in the youngest to 2·5% in the oldest group, compared th 3·2% among controls. The relative risk for Bf F1 + S1 ·4 in patients 10 years or younger.

Age-dependent relative risks in IDDM have peviously been orted[2] for HLA Bw15. In our own series relevant HLA data available for 148 of the IDDM patients. There is no signifint difference in the frequency of B8 for patients in the vars age categories, but for Bw15, 14% (13/93) are in the 0–10 group and 9·3 (5/54) are in the 10–20 age group. The mbers are small but the trend is in the same direction as that Dausset.[2]

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The rapid decline in the frequency of Bf F1 with increasing age of patients with IDDM is compatible with a viral stimulus for initiating the disease process. If Bf F1 is in positive linkage disequilibrium for a susceptibility allele then young children carrying the F1 allele are more likely not to resist an infection, with consequent development of autoimmune responses and islet-cell destruction. With increasing age the development of partial immunity in the child population may reduce the strength of the interaction between the precipitating agent and factors linked to the susceptibility allele.

In our laboratories we have demonstrated also a high frequency of lymphocytotoxins (LCs) in sera of patients with IDDM. There is a strong association between the presence of LCs and HLA type: they were present in 37% of patients homozygous for either B8 or Bw15 compared with 16% in those not homozygous for either. A similar relationship is present for LCs and Bf factors: they were present in 38% of patients with the Bf F1 factor compared with 17% in patients without this factor. Since in our data Bf F1 is in strong negative disequilibrium with HLA-B8,[3] this relationship between LCs and Bf F1 is not due to the similar relationship between LCs and HLA-B8.

Islet-cell antibodies are also more frequent in sera of IDDM patients who are HLA-B8,[4] and this relationship is even stronger in those patients in whom islet-cell antibodies have persisted for 5 years or more. We are now investigating the relationship between the frequency of F1 and S1 and age of the patient, together with the significant association between F1 and the presence of lymphocytotoxins, suggest that Bf typing will be of increasing importance in differentiating various factors in the aetiology and progress of insulin-dependent diabetes.

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